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The Fungal Community

Its Organization and Role in the Ecosystem



Third Edition

edited by
John Dighton
James F. White
Peter Oudemans



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Preface

The third edition of *The Fungal Community* has been compiled by a new set of editors. The three of us were impressed with the quality and content of the previous two editions and hope that we have matched the work of George Carroll and Don Wicklow in this new volume.

The aims and objectives of this volume are explained in our introductory chapter, but in brief, we have tried to address some of the current discussions in ecology (diversity and function, scaling issues, disturbance, invasive species) from a fungal perspective. In order to be able to address these issues, we need appropriate techniques to identify fungi, determine their abundance, determine their associations among themselves and other organisms, measure their individual and community function, and be able to scale these measures from the microscopic level of the individual hyphal or fungal cell through local to landscape and ecosystem levels. The chapters of this edition have advanced toward addressing these aspects of mycology and beyond, but they are by no means all-encompassing. We apologize if some areas are missing, but the size of this volume speaks for the complexity and dimension of aspects relating to the functional role of fungal communities in ecosystems, and this book would be overwhelmingly large if we included more. There is a huge body of literature in both mycological and ecological journals that pertains to the themes contained herein, and we praise our authors for their extensive bibliographies, which will steer interested readers to some of the most recent publications in their specific areas.

Editors

John Dighton is a professor in the Institute of Marine and Coastal Sciences (Cook College) and Biology Department (Camden) and director of the Pinelands Field Station of Rutgers University. He is the author of *Fungi in Ecosystem Processes* (2003) and author or coauthor of over 100 journal articles on soil ecology and mycology. He is a member of the British Mycological Society, the Mycological Society of America, and the Ecological Society of America. John Dighton is on the editorial board of *Mycological Research, Soil Biology and Biochemistry,* and *Bartonia*. He received his B.Sc. degree as an external student of London University in botany and zoology, an M.Sc. degree in ecology from Durham University, and a Ph.D. in ecology from London University.

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Peter Oudemans is an associate professor in the Department of Plant Biology and Pathology at Rutgers University and is stationed at the Philip E. Marucci Center for Blueberry and Cranberry Research and Extension. His research goals are aimed at the biology and control of fungal diseases of the blueberry and cranberry. To accomplish these goals, he uses remote sensing and geographic information system methodologies to detect, map, quantify, and track plant pathogens, as well as other interacting factors that influence crop quality and yield. In addition to research, he teaches classes in plant pathology and mycology, as well as a colloquium course entitled “Agriculture in the Pinelands.” He received his training at the University of New Brunswick (B.S.), the University of Guelph (M.S.), and the University of California (Ph.D.), as well as postgraduate training at Duke University and the University of Kansas.

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We thank all our authors for their contributions and for helping to make the process of editing this volume as painless as possible. We hope that we have not put too much pressure on any of them. We also extend a debt of gratitude to Magnus Anusiem, Vanessa McCowan, and other members of our laboratories who have helped us with collating and indexing this book.

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THE THOUGHTS BEHIND THIS VOLUME

Why is it that it took three people to edit this third edition of *The Fungal Community* when it has so eloquently been done by two people in the past? Are we so much less competent? Perhaps so, but we would like to think that in the interval between the last edition and this a number of new technological advances have been made, allowing us to have more tools, or toys, to play with to study fungi and fungal communities, and we are not all familiar with all methods available. Thus, among us, we hope that we have assembled a mixture of authors who can address some of the questions regarding the observations, characterizations, and functional attributes of fungal assemblages and their interactions with both the environment and other organisms. In addition, the ecological literature has expanded to ask questions of global and local biodiversity, highlight the problems of exotic species, reopen the debate about diversity and function, and become more aware of the functional rather than taxonomic methods of classification.

All of these factors impact our way of looking at communities, interactions between species, and community function. New tools, particular molecular methods of identification of individuals and the phylogenetic relations of these individuals, and the molecular identification of functional regulators allow us to investigate the functional aspects of individuals and groups of individuals (communities) in different ways than in the past.

The speed at which these new technologies are evolving makes it difficult for one individual to be able to be conversant with the details of all methods, their utility, limitations, and applications.

Thus, we hope that among the three of us we may be able to show a degree of competence in some of the subject areas we encompass in this volume. We have attempted to address a number of current ecological concepts and approach the concept of fungal communities from an ecological perspective, rather than a fungicentric view. We hope that the melding of ideas, methods, and results promoted by our contributors will point to directions in which mycology should proceed in the future.

LITTLE AND LARGE: SCALE IN PERSPECTIVE

Fungi are regarded as microorganisms. Their individual components, hyphal filaments, are microscopic. However, by their nature of excreting enzymes, low-molecular-weight organic acids, etc., they have an influence on a larger sphere than the space occupied by their biomass. Together with the extensive growth of hyphae through the substrate colonized by the fungal mycelium (Rayner, 1998), information (carbon and nutrients) can be effectively translocated by them (Jennings, 1990; Wells and Boddy, 1995; Wells et al., 2001). Thus, the influence of fungi extends to a macrolevel of space (Rayner, 1992). In conjunction with vascular and other plants, the influence of fungi can extend to the landscape level. How can we translate among scales of resolution influenced by fungi? How can we translate the activities occurring at the surface of individual hyphae into macroscale effects. How can we account for macroscale perturbations and heterogeneity on physiological and biochemical changes in the fungal hyphae at the cellular level? The influence of scale and heterogeneity in the ecosystem is a confounding factor in our attempts to understand ecology and community functioning. However, these factors are inherent in ecosystems, and we need to be able to identify them and develop tools to use the unique properties that these variables provide to ecosystems. How are ecologists overcoming these problems? Can we apply the same ecological principles to investigate fungal communities at each of these scales, and how does observation at each scale allow us to interpret what is going on at the scale above or below that which we are observing? This is one of the more intangible questions of ecology, but it allows us to think about experiments and observations required to transcend these scales (O'Neill, 1988; O'Neill et al., 1991; Friese et al., 1997). This subject runs throughout this volume and is introduced in Chapter 1 by Morris and Robertson, where they relate the function of fungi and fungal communities at different scales of observation or measurement.

WHAT IS A FUNGAL COMMUNITY?

What do we really mean by a community? We may take the definition of Whittaker (1975): "an assemblage of populations of plants, animals, bacteria and fungi that live in an environment and interact with one another, forming together a distinctive living system, with its own composition, structure, environmental relations, development and function." Using this definition, it seems impossible to consider the structure and development of fungal communities without investigating their interactions with other organisms and the environment. A good discussion of the properties and dynamics of communities can be found in Peter Morin's book *Community Ecology* (1999). A view of fungal communities, their identification in relation to plant phytosociological taxonomy, and a call for mycol-

ogists to become more precise in their definitions of fungal communities come from Chapter 2 by Hawksworth and Mueller, and later from Chapter 13 by Tuininga. Fungal communities are inextricably related to communities and populations of plants and animals in the ecosystem. The effect of fungi on other organisms and the effects of other organisms on fungi are important aspects of community analysis in any ecosystem. In this respect, we have chapters that describe interactions between fungi and plants (Chapter 9 by Van Bael et al. and Chapter 21 by Rudgers and Clay, for endophytes; Chapter 22 by Bever and Schultz, with plant communities; Chapter 27 by Zuccaro and Mitchell, for seaweeds), fungal interactions with animals (Chapter 28 by Ruess and Lussenhop, for invertebrates; Chapter 29 by Trappe and Claridge, for vertebrates), and interactions with microbial communities in biological soil crusts (Chapter 6 by Belnap and Lange). These interactions encompass the broad spectrum of competition, predation, mutualism, commensalism, and amensalism found in all communities.

IDENTIFICATION AND CHARACTERIZATION OF A FUNGAL COMMUNITY

How do we characterize communities of fungi? Methods required to identify individuals within the community include classical methods of direct observation (largely restricted to the identification of fruiting structures), culturing methods (linked to direct observations), and molecular/biochemical methods. Are these methods mutually exclusive? Is each of the methods robust? For example, the identification of species and communities based on fruiting structures (mushrooms) provides information only on mushroom distribution and abundance, saying little about the abundance, distribution, and interactions of other parts of the mycelium, which is usually the functional component of the organism. Isolation techniques are media dependent. How many replicate media are required to adequately extract and identify all species that are present in the environment under study? Many times a single medium or sometimes two media are used. Is this adequate?

Some of the classical methods for the study of fungal communities are discussed by Schmit and Lodge in Chapter 10. More recently, molecular methods of DNA extraction and comparison with known DNA libraries have become in vogue. However, how adequate and robust are these methods when used in isolation? Because the methods have become more available and easier to use, is sample preparation before extraction influenced by quality control protocols? Can extraneous DNA amplified from inadequately prepared source material confuse the interpretation of molecular profiles obtained? Are all molecular profiles checked against DNA extracted from isolated fungi that have been identified by classical techniques? Is this degree of rigor really required, or can we get by with a quick and dirty global DNA extraction? These questions are addressed in Chapter 11 by Bidartondo and Gardes. In order to determine who is where, there is also a need to identify where an individual organism and population reside. Molecular tools are being used to allow us to identify where similar and dissimilar genetic information in the same species exists, identifying population demography. These methods are described by Zhan and McDonald in Chapter 12.

Terminology used to describe interactions between organisms and populations is complex in all fields. In fungal ecology, the terminology is probably as complex as in other disciplines, or even more so. Terminology has changed over time, and common terminology has been used to mean different things by different people (Cooke and Rayner, 1984). In Chapter 13, Tuininga attempts to unravel some of these complexities and suggests a revision of the terminology that may lead to less ambiguity in the future.

Descriptions of fungal communities are represented here at a variety of spatial scales: from biome; γ-diversity (Chapter 4 by Kis-Papo, Chapter 5 by Hyde et al.) through landscape; β-diversity (Chapter 3 by Bärlocher and Chapter 6 by Belnap and Lange) to local; and α-diversity (Chapter 27 by Zuccaro and Mitchell). For discrete, mobile organisms, changes in resources usually lead to a redistribution of individuals and populations to areas or niches that are most suitable for their existence and controlled by birth, death, and rates of emigration and immigration. In addition to spore or propagule dispersal, nonmobile organisms, such as fungi, frequently occupy niche space by the growth of single individuals and compete with other individuals by chemical warfare. Do the dynamics of these communities follow the same rules of density-dependent regulation and Lotka–Volterra models of species interactions (see Morin, 1999)?

The development of communities depends also on time. During fungal community development the nature of the resources available to the fungi also changes. This is explored in an evolutionary context by Van Bael et al. in Chapter 9 and over the time course of decomposition (Chapter 8 by Ponge). The relative influences of different factors affecting the outcome of intraspecific interactions, leading to changes in community structure, are known as assembly rules (Drake, 1990) and are discussed here, in reference to mycorrhizal fungi, by Jumpponen and Egerton-Warburton in Chapter 7. These temporal changes in community structure of nondiscrete, nonmotile organisms may differ from those classically thought of by ecologists. In the decomposer community, for example, niche breadth is changed over time by the fungal community occupying that niche (Swift, 1976), leading to a sequential change in fungal communities occupying the same resource (Ponge, 1990, 1991; Frankland, 1992, 1998), as discussed here by Ponge (Chapter 8). Are assembly rules for fungal communities the same as for other plant and animal communities?

FUNCTIONALITY IN FUNGAL COMMUNITIES

Characterization of the fungal community can tell us the nature of the components of the community; for example, information can be gained by molecular profiling. But what degree of functional information is gained from physiological and biochemical profiling, such as BIOLOG (Winding, 1994) of FUNGILOG (Zak et al., 1994; Dobranic and Zak, 1999)? Do these methods actually provide us with the true function of the community as it is *in vivo*, or are community constituents influenced by the media upon which they are isolated and grown prior to the biochemical profiling? An alternative to thinking in classical Linnean taxonomic terms is to think in terms of guilds (Root, 1967) or functional groups, where organisms are grouped on the basis of their functional attributes, irrespective of their taxonomic status. How much of the function of fungal communities can be explained in terms of, for example, enzyme expression (see Chapter 16 by Lindahl et al. and Chapter 17 by Sinsabaugh)? These biochemical changes in the environment created by the presence of fungal hyphae are at the local or microscale, despite the fact that they can be integrated over larger scales and have landscape effects (Dighton, 2003). As fungi are microorganisms, detection of changes in the local environment under the influence of an individual hyphum is limited because analytical techniques are usually designed for measures of changes in the chemistry of the environment (Dighton et al., 2001). Turnau and Kottke (Chapter 14) and Czymmek (Chapter 15) provide methods where the influence of single fungal hyphae may be measured, and Lindahl et al. (Chapter 16) describe influences at the local scale of mycorrhizal root surfaces.

At the larger scale of resolution, Sinsabaugh (Chapter 17) shows how the interactions between fungi and bacteria can lead to larger changes in soil enzyme activity and expression, acting in a true community sense. Eric Hobbie (Chapter 18) introduces us to the use

of stable isotopes to follow the transformations of elements as they cycle through ecosystems via fungal communities. These methods are becoming more frequently used in ecosystem studies, and their application to understanding the functional role of fungi in the ecosystem process is an important subject for the future. How does the diversity of a fungal community relate to its function? The debate regarding biotic diversity and the functioning of an ecosystem has been present in the ecological literature for a number of years (Naeem et al., 1994; Tilman, 1999, 2000; Naeem, 2002). However, the relevance of diversity in the functioning of fungal communities has been less well documented. Based on the pioneering studies of van der Heijden et al. (1998a, 1998b), Baxter and Dighton (Chapter 19) discuss the role of diversity and species composition in the function of ectomycorrhizal fungal communities as an example of the application of community manipulations previously common only in plant and animal community studies.

FUNGAL COMMUNITY INTERACTIONS WITH OTHER ORGANISMS

Fungi do not occur *in vacuuo* in the environment. Their evolution with plant species can be traced back in the fossil record to the time when the first land plants emerged (Pirozynski and Malloch, 1975) and probably before that time. They are associated with animals both as pathogens and in trophic interactions. We have considerable evidence that fungi are closely associated with bacteria, especially in soil and aquatic ecosystems (Berthelin and Leyval, 1982; Berthelin et al., 1995). We have highlighted here a variety of interactions between fungal communities and other organisms in the environment. In Chapter 20 Kobayashi and Hillman discuss the interactions of fungi with bacteria and viruses. Chapters 24 through 26 and Chapter 35 discuss the evolution of and consequences of fungal interactions with plants in the form of fungal endophytes.

Since the last publication of *The Fungal Community*, much has been learned about how asymptomatic fungal endophytes alter the ecology of plant hosts. Rudgers and Clay (Chapter 21) provide an overview of the state of the ecological knowledge of these interactions and provide the context for succeeding chapters. In Chapter 9 Van Bael et al. discuss research and concepts regarding fungal endophytes of tropical plants and demonstrate the widespread importance of endophyte–plant interaction. In Chapter 26 Bacon and Lyons discuss competition between endophytes, the production of secondary metabolites, and their consequent impact on herbivores. Schardl and Leuchtmann (Chapter 24) and Bischoff and White (Chapter 25) demonstrate the diversity in clavicipitalean fungi and discuss how these fungi have evolved as symbiotic associates of plants. Taken collectively, this group of chapters provides a snapshot of the current knowledge of the ecology of aerial plant endophytes.

Pathogenic fungi are best known for their effects on agricultural crops. In natural ecosystems, the influence of fungal pathogens is less clearly defined. Similarly, the role of mycorrhizae has traditionally been that they enhance plant growth by facilitating nutrient uptake. Bever and Schultz (Chapter 22) and Hansen and Stone (Chapter 23) discuss the interactions between these functional groups of fungi and the regulation of plant community composition from a mycorrhizal and pathogenic perspective, respectively. Especially from a pathogenic perspective, there is much debate in the ecological literature regarding the invasiveness of exotic plants and animal species. The role of exotic plants on fungi and exotic fungi on plants has recently been highlighted in the ecological literature (Rossman, 2001; Wingfield et al., 2001) as a cause of concern for agriculture and forestry.

Interactions of fungi with animals are shown from a trophic perspective in both invertebrates (Chapter 28 by Ruess and Lussenhop) and vertebrates (Chapter 29 by

Claridge and Trappe), as fungi can form a major part of the diet of a range of animal species. In addition, the role of animals as vectors for fungal propagules may be important for mycorrhizal colonization (Klironomous and Moutoglis, 1999), distributing fungal pathogens to root pathogens (Doube et al., 1995), regulating gut-inhabiting pathogenic nematodes (Faedo et al., 1997), facilitating colonization of plant litters during decomposition (Moody et al., 1996), and helping mycorrhizal establishment of plants during primary and secondary succession (Allen, 1987). Some of these nontrophic interactions are discussed in Chapter 30 by Trappe and Claridge.

HUMAN IMPACTS ON FUNGI

As a result of our impacts on the environment, we are creating pollution in a variety of forms, possibly changing the climate and weather patterns and altering the landscape by our agricultural and urbanization activities. Fungi are affected by these activities, as are a range of other organisms. However, along with other microorganisms, fungi have the capacity to interact with a number of pollutants, reduce their toxicity, and in other ways adapt and modify the effect of these pollutants.

Swift (Chapter 31) introduces this section with a broad overview of human interactions with fungi. In order to appreciate the ways in which fungi can overcome the stresses of climate change and pollutants, we look to the ways in which fungi adapt and function in other stressed environments. Hence, Chapters 32 through 34 by Wainwright, Zak, and Rodriguez et al., respectively, discuss the adaptations of fungi in oligotrophic, desert, and other stressed environments. Zak discusses the heterogeneous distribution of fungi in desert ecosystems in relation to α- and β-diversity and in correlation with patches of vegetation. The adaptations of and changes in fungal species and communities because of stress are discussed in Zak's chapter also, and Belnap and Lange (Chapter 35) and Treseder (Chapter 36) discuss the potential interactions of soil crust and mycorrhizal communities to climate change.

The effects of pollutant chemicals and the effect that fungi can have on altering the pollutants are discussed in terms of heavy metals (Chapter 37 by Fomina et al.), radionuclides (Chapter 38 by Zhdanova et al.), and acidifying pollutants (Chapter 39 by Lilleskov).

Recent interest in land use change has suggested that conversion of natural ecosystems to agriculture could severely impact carbon budgets around the globe (Houghton, 1994; Howard et al., 1995). As a result, there is considerable interest in budgeting the potential carbon sink offered by soils in the form of protected organic matter (Miller and Jastrow, 1990, 1992) and other functions that may be partially mediated by fungi. Hence, Golovko and Ellanska (Chapter 40), Stromberger (Chapter 41), Durall et al. (Chapter 42), and Rizzo et al. (Chapter 43) discuss the changes that may be brought about in the fungal community by changes in land use practice and the possible effects on ecosystem processes. Of particular importance is the role of exotics in influencing community composition. The easier it becomes to transport organisms around the world, the more influence fungi may have on the structure and development of new existing plant communities (Rossman, 2001), as discussed by Rizzo et al. (Chapter 43).

BIODIVERSITY AND CONSERVATION

The concept of fungal conservation was first highlighted by the surveys of Arnolds (1988) suggesting a decline in the abundance of ectomycorrhizal basidiomycete fungi in the

Netherlands. As a result of these observations, he initiated the first red data list for fungi (Arnolds, 1989) and thus paved the way for future fungal conservation initiatives (Arnolds, 1997). How do we adequately assess the diversity and abundance (rarity) of often cryptic or ephemerally evident organisms? For the macrofungi it may be considered an easy task, as they produce macroscopic fruiting structures that can be identified and enumerated. In an ongoing conservation survey of macrofungi in the U.S. Pacific Northwest, this task is anything but simple. This survey is using large (5.5 km) grids to sample a large area of land. Surveyors are finding that some species do not fruit each year, some species are hypogeous requiring extensive time to find them in the search area. It is estimated that some 20 plot years of survey would be necessary to obtain an accurate representation of the fungal species community (Molina, personal communication). The predicted time required to assess the community composition of these fungi was determined by looking at long-term data sets of collections in the area and identifying surrogate measures to predict diversity. Many areas of the world do not have such databases on which to mount a predictive model, so our quest for global mapping of fungal diversity is a daunting task. These limitations must be kept in mind when evaluating the survey data presented in Chapters 4 and 5. Plant ecologists have a head start on mycologists in regards to the identification and mapping of plant species and communities. What tools do the ecologists have that the mycologist could use? Recent advances in the interpretation of aerial and satellite images (Hughes et al., 1998) may be a possible way to estimate diversity, especially if we know correlations between fungal species and vegetation type, and allow us to identify areas that are under threat and require conservation (see Chapter 44 by Watling).

CONCLUSIONS

It has not been possible to include all aspects of fungal communities, fungal ecophysiology, and biogeography in this volume. However, we hope that the selection of chapters we have assembled provides insights into the complexity of studying fungal communities and the importance they may have on broad, ecosystem, and landscape scales, as well as on more local scales. The authors were instructed to be as controversial as possible so as to generate a series of questions or sow the seeds of thought in future generations of mycologists. Given the estimate of more than 1.5 million fungal species possibly in existence in the world (Hawksworth, 2001), it is impossible for us to generalize about the physiology and ecology of fungi. We will see in the following chapters immense diversity in the functioning of fungi, their associations with other organisms in the community, and their role in regulating ecosystem processes. Given that we understand little about the ecology and physiology of a fraction of the possible total number of fungi, we feel there is plenty of room for new mycologists to continue the investigations into these somewhat unique organisms.

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Section 1

Structure of Fungal Communities

1

Linking Function between Scales of Resolution

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1.1 INTRODUCTION

There has been an increasing focus on the importance of spatial scales in ecology. The focus is twofold in that an understanding of spatial and temporal scales is essential both for describing the distribution of organisms and for understanding the roles of organisms in ecosystem function. Issues of scale may be especially important for understanding fungal contributions to ecosystem dynamics as fungi play essential roles in nearly every aspect of ecosystem development, stability, and function. Fungi and fungal community structure play major roles in determining both above- and belowground biodiversity within ecosystems (e.g., Van der Heijden et al., 1998a, 1998b; Bever et al., 2001; Klironomos, 2003). Improved understanding of the spatial and temporal activity of fungi in ecological systems is necessary for evaluating their specific roles in ecosystem function.

Fungi control many regulatory steps in ecosystems. For example, as saprophytes, they control the rate at which organic matter is returned as inorganic nutrients available for plant uptake. As mutualists, they provide nutrients and water to plants to increase net primary productivity. As pathogens, they cause mortality and affect community composition and turnover. Yet we know relatively little about the size and component parts of the fungal network that contribute to each function.

Waksman (1916) suggested that "the question is not how many numbers and types of fungi can be found in the soil, but what organisms lead an active life in soil." This

fundamental question is still relevant today. Ecosystem function is not governed by the species of fungi present, but by the role that each fungus plays in carrying out certain tasks and the rates at which these tasks are accomplished. Future research needs to link diversity and function. Much of the current literature that addresses microbial community dynamics does not differentiate between the relative contributions of fungal communities and bacterial communities. We thus have a poor understanding of both the specific role of fungi in general and the specific roles of individual species for most ecosystem processes in which they participate. By definition, this is a scalar issue: fungi act on individual molecules at microscopic scales, yet aggregate effects are felt at ecosystem and landscape scales. In this chapter we first present ecological questions that mycologists are not now adequately addressing and then focus on the tools needed to adequately evaluate soil fungal communities.

1.2 ECOLOGICAL SCALE

1.2.1 Linking from Molecules to Individuals

Molecular signaling plays a large role in directing the life cycle and functions of fungi. Evaluating the response of fungi to external stimuli, including dormancy, germination, resource acquisition, sporulation, and dispersal, requires an understanding of the molecular cues that signal appropriate timing for each of these events. For evaluating soil fungi, the cues for dormancy or for germination are a sufficient start for tying molecular level processes to individual behavior. More important, perhaps, are the cues that signal positive interactions, such as the formation of mycorrhizas, and negative interactions, such as staving off attacks by pathogens. Advances have identified some of these cues, e.g., alterations in nutrient content, light, aeration, temperature, pH, and activity of phenols and polyphenoloxidases (Andrews and Harris, 1997), but the fine-scale work to examine what promotes these activities in the natural environment lags behind laboratory work that may not adequately represent *in vivo* conditions. Simplistic approaches are valuable for identifying potentially important interactions but necessarily ignore complex species–species interactions such as multitrophic signals in the rhizosphere. These can be of great importance, a consequence of the long coevolutionary history among rhizosphere organisms (Phillips et al., 2003). Knowledge of the extent of these molecules exist and of the processes they control is necessary for evaluating rhizosphere control points and, more importantly, for interpreting consequences of anthropogenic disturbance for belowground communities.

Molecules used for food acquisition are as important as signaling molecules. Measurements of exoenzymes have begun to provide important information on the activity of soil microfungi and the resources that they are consuming, but there is yet little linkage to the types and numbers of fungal species that produce the enzymes. The reduction of competition for food resources is also mediated by the production of antimicrobial or antifungal compounds that affect species distribution at small scales. Of particular interest are the molecules used by ectomycorrhizal (EM) fungi for capturing nutrient resources. For example, predation upon live collembolan (Klironomos and Hart, 2001) or dead nematodes (Perez-Moreno and Read, 2001) in soil by EM fungi allows for a much more direct route of nutrient acquisition. These pathways are likely driven by enzymatic activities that can be detected at the molecular level in soils. Determining whether other fungi are capable of deriving nutrients directly from organisms in the soil food web is necessary to complete linkages in nutrient cycles, fully evaluate the impacts of species loss, and allow for an understanding of the evolution of these traits and their relevancy in terms of overall nutrient cycling in soils.

Identifying the molecules that affect and are affected by fungi is essential. Determining the degree to which molecules influence multiple trophic levels or affect synergistic activities is also important. Identifying the spatial scale at which these molecules work, their patterns of temporal production, and their longevity is significant for evaluating the impact of these molecules on overall community dynamics.

1.2.2 Linking from Individuals to Communities

Overall, the diversity of soil fungi is immense, with current projections at 1.5 million species (Hawksworth, 1991). The unique genetics of fungi, including homo- vs. heterokaryotic organisms, allow for molecular control of mechanisms that differ from other organisms and allows genetic diversity to be preserved and increased in unusual ways. Population characteristics of fungi are influenced by their unique genetics. For example, the short dispersal distances of fungi would suggest that there might be low genetic diversity within populations, yet research by Vandenkoornhuyse et al. (2001) suggests that this may not be the case: specific fungal groups may have a much greater intrapopulation genetic diversity than interpopulation diversity. Müller et al. (2001) detected greater diversity within populations for endophytes than for saprophytes on the same tissue. Villeneuve et al. (1989) found that mycorrhizal species richness is relatively constant along a gradient of environmental disturbance, while saprophytic fungal diversity decreases along the same gradient.

High genetic diversity within populations may be instrumental to the ease with which fungi have evolved mutualistic relationships in multiple groups. The extremely high levels of variation in small arbuscular mycorrhizal (AM) populations suggest that mechanisms for recombination have been underestimated in fungi and recombination rates may actually be enhanced by changes in environmental conditions to which fungi are exposed (Vandenkoornhuyse et al., 2001). This has been extremely difficult to study in field trials. Laboratory studies are now beginning to confirm that genetic diversity of fungi in soil environments is much higher than fungal diversity of organisms found in laboratories (Castelli and Casper, 2003). Greater ties among population dynamics such as genetic structure, spatial distribution of individuals vs. hyphal networks or spores, and the relative age structure of populations would contribute greatly to defining the role of individual species in community interactions.

Increased understanding of genetic diversity in soil fungi is also essential to evaluate the degree to which there is true functional redundancy. While great strides have been made in identifying organisms, especially since the increased availability of molecular tools, tying specific organisms to specific processes in the complex environmental matrix of soil is still lagging (Gray et al., 2001). Examinations of AM and EM fungi as a functional group have indicated that mycorrhizal fungi respond directly to environmental cues, independently of their plant host (Allen et al., 1995). Research indicates that there is high functional diversity in mycorrhizal fungi within and across habitats, and should there be loss of fungal species, there will be a significant shift in how plants acquire resources in specific habitats. More studies that tie genetics to function are necessary to evaluate the degree to which loss of genetic diversity will affect the resistance or resilience of ecosystems following global climate change. Identifying individual species and responses to environmental cues is essential to evaluating the roles and interactions of fungal species in terrestrial communities.

1.2.3 Linking from Communities to Ecosystems

Fungi play multiple roles in terrestrial communities as saprotrophs, predators, and pathogens and as mutualists of photosynthetic organisms (lichen, mycorrhizas). Fungi can be

endophytes on leaves that fall from trees and then become part of decomposer communities. They are key components of soil food webs as consumers, predators, pathogens, and decomposers. Few would argue that their contributions to community dynamics are not important to the organization and structure of terrestrial systems. Exploring the interactions of fungi within the fungal community and their role in determining plant communities, especially as decomposers and mutualists, has highlighted the fact that fungi are intimately involved with components of energy acquisition and distribution.

Fungal pathogens can play a large role in maintaining plant species diversity. Pathogens can influence the success of a given species by allowing it to coexist with other species (Westover and Bever, 2001), or pathogens can cause the loss of a species by decreasing its competitive ability, allowing its replacement during succession (Van der Putten and Peters, 1997), during competition, or following disturbance. These relationships can be difficult to detect, as some interactions among pathogens and synergisms with mutualists can depend on life stage (Smith and Read, 1997). Pathogens can also play a role in tree species diversity and in the spatial distribution of species (Packer and Clay, 2000; Reinhart et al., 2003). Mortalities of black cherry seedlings were very high under soil collected from under black cherry, but not from 30 m away, due to a pythium species that prevented seedling establishment. This inhibition was alleviated when black cherry was introduced in an area without pythium.

A great deal of research has addressed the impacts of mutualists on plant community structure. Plant diversity is promoted by mutualists that supply nutrients to plants that would otherwise be poor competitors. Some of this research suggests that diversity can be increased only if AM fungi are heterogeneously distributed or if benefits to plant species differ (Jordan et al., 2000). Differences in the efficiency of resource capture by mycorrhizal fungi and the resultant impact on plant growth have been demonstrated many times (Van der Heijden et al., 1998a; Klironomos, 2003). The impact of mycorrhizae on its host can range from that of a parasite to that of a mutualist. The consequence is differential impacts on host species with concomitant effects on aboveground species diversity.

Little attention has been focused on the impact of belowground diversity on aboveground function. Baxter and Dighton (2001) found that increasing fungal diversity decreased shoot growth of grey birch and increased mycorrhizal root length. This suggests a decrease in benefit for plants with increased mycorrhizal diversity. In contrast, Klironomos et al. (2000) found an asymptotic increase in net primary production (NPP) with the addition of belowground species. The increases in plant productivity with added aboveground diversity found by others, such as Tilman et al. (1997), were not mirrored by an increase in plant productivity with increased belowground diversity. The addition of only two mycorrhizal species saturated the productivity curve.

In addition to impacts on aboveground plant diversity, mycorrhizal fungi can also influence other communities such as insects. Gange (2001) found that a single mycorrhizal fungi decreased larval survival and biomass of the root-feeding black vine weevil, whereas colonization by two mycorrhizal fungi did not. Similarly, Gange et al. (1994) demonstrated that the presence of mycorrhizae on the roots of *Taraxacum officinale* decreased the number of black pine weevil larvae feeding on the roots. Both ecto- and endomycorrhizal species have been reported to protect plant hosts from pathogenic attack (Azcon-Aguilar and Barea, 1992).

1.2.4 Linking from Ecosystem Scales to Global Scales

Read and Perez-Moreno (2003) have suggested that mycorrhizal fungi may provide a crucial link between communities and ecosystems. The relationship integrates above- and belowground dynamics as the response variable for nutrient cycling and decomposition

and is a rate-limiting step that can influence both net primary productivity and tissue quality. Cornelissen et al. (2001) compared plants of known functional and mycorrhizal type and found that mycorrhizal strategies are linked to productivity and litter turnover. The physiological potential of each mycorrhizal group (AM, EM, ericoid) may allow for the development of a mechanistic understanding of distinctive plant communities across local to regional scales.

Modeling allows a mechanism for linking ecosystem level processes with real-world scenarios. These models are useful for predicting changes in global scale patterns due to changes in ecosystem level processes and for understanding the impact of abiotic change on biotic communities and feedbacks between the two. Fungi have been incorporated into these models as components of nutrient turnover, but rarely as more than a black box. Because the most important indicators of microbial activity at the global scale are moisture and temperature, the role of fungi as decomposers is often included as a simple rate function or as a component of organic matter turnover. These models have capabilities necessary for predicting changes to nutrient turnover under differing scenarios of global climate change, land use change, or alterations to system management, but are not adequate to evaluate changes to ecosystem components if alterations result in changes in fungal species that affect ecosystem energy acquisition or species diversity.

Hunt and Wall (2002) specifically modeled the effect of species loss on net primary productivity and found that the deletion of only two groups, saprophytic fungi and bacteria, caused large changes in net primary productivity. This suggests that as a group, fungi are not redundant, nor are they functionally interchangeable with bacterial decomposers. Much would be gained from including fungi as a group in modeling efforts, but first, specific model parameters must be created and evaluated, and specific values for contributions of mutualists, saprophytes, pathogens, and predators need to be derived. To achieve the goals of linking individuals to communities and to link these roles in a quantitative fashion to ecosystem dynamics require tools appropriate to different scales.

1.3 PHYSICAL SCALE

Fungi are spatially structured in soils in response to a number of biotic and abiotic features (Ettema and Wardle, 2002). At the smallest scales, fungi respond to soil pores, aggregates, particulate organic matter, and fine roots. They are also structured in response to vegetation patterns such as size, spacing, root distribution, and the distribution of vegetative resources such as exudates, leaf litter, stem flow, and throughfall. At larger spatial scales, fungi are structured by soil type, land use, topography, and microclimate. At global scales, they are affected by climate and by anthropogenic disturbances such as pollutants. Integrating across physical scales is necessary to integrate fungal dynamics across ecological scales. The current approach to understanding fungal ecology is limited by the techniques and approaches currently available.

1.3.1 Linking from the Microscale to the Plot Scale

At the microscale, current methodology for sampling fungi is limited. Evaluating mechanisms by which fungi acquire their resources at a scale relevant to the organisms themselves has been difficult in the field under natural conditions. The recent development of techniques that allow for the *in vitro* evaluation of organisms under laboratory conditions on native substrates is providing data that will allow us to more easily transfer studies from laboratory to field situations. Resources can now be tied to the organisms responsible for decomposition in such a manner that changes in chemistry and organisms can be

followed simultaneously. For example, litter carbohydrate availability has been tied to the advancing mycelial front by microscopic Fourier transform infrared spectroscopy (Dighton et al., 2001). This process allows fungal succession to be plotted against specific changes in substrate chemistry. Ultimately, fungal succession can then be tied to process level mechanisms.

In addition to determining the interface between resources and organisms, it is also essential to determine the location of the substrate and the distance over which nutrients travel. Gaillard et al. (1999) demonstrated changes in microbial heterogeneity by following ^{13}C and ^{15}N concentrations in soil. Movement of materials up to 4 mm away from the labeled substrate was attributed to transport through fungal hyphae growing on the substrate. This distance begins to define what is now considered the detritusphere and should begin to suggest the size of the feeding zone relevant to fungi. Developing techniques for evaluating the relationship between hyphal development, nutrient acquisition, and transport distance should allow mechanistic investigations of decomposition dynamics to be linked to species diversity. Quantitative analyses of mechanisms by which fungi acquire resources and participate in nutrient cycling are necessary to link diversity and abundance to specific ecological roles.

There are few approaches available to study intact fungal communities. Culture work only allows for isolation of individual fungi, and few organisms can be manipulated this way. Community studies using this technique provide little understanding of the role of fungal biomass or diversity in soil. Collecting fungal hyphae or spores from soil cores for cultures fails to preserve hyphal networks, destroys linkages between fungi and other organisms, and obscures the extent to which the fungi affect ecosystem function in soil systems. Measurements of hyphal lengths can indicate the presence of a fungus at some time in the past, or they can indicate the presence of an active fungus, depending on the techniques used. In either case, such measurements do not indicate the activity of the organism, its age, or its identity.

Not all hyphae are equal in function, contribution to soil dynamics, or community structure. Fungal hyphae can be differentiated based on a number of characteristics. Prior to the development of molecular techniques, hyphae were distinguished based on physical characteristics, and this provided information on a number of interactions of specific hyphae in soils. For example, differentiating hyphae based on color alone increased the understanding of the differential preference of fungi as a food source for microarthropods (Klironomos and Kendrick, 1995a). Lab feeding trials had suggested that collembolan prefer mycorrhizal fungi, yet field observations of coloration led Klironomos and Kendrick (1996) to suspect a larger role for pigmented fungi on decaying litter, which was confirmed by more elaborate feeding trials. This illustrates the degree to which our understanding of small-scale dynamics can be obscured by moving organisms out of their native soil matrix.

The rate at which hyphae are produced and retired in soils has been poorly quantified. Recently, Staddon et al. (2003) detected hyphal turnover rates for AM fungi suggesting that extraradical hyphae turn over on average every 5 to 6 days. Turnover this rapid makes hyphae a very rapid conduit by which C is supplied directly to belowground systems from plant photosynthesis. Additionally, Rillig et al. (2003) found correlations between AM mycoproteins and a soil C pool of significant size and relatively slow turnover rates. As hyphae and products of fungal growth have significant impacts on local soil C pools, they should be included in examinations of global C cycles.

Fungi as saprophytes, mutualists, and pathogens are involved in hyphal networks that connect them to nutrient sources and water, form bridges between plant species, participate in sporulation, form aggregates, and provide for invasions of uncolonized areas. Fungal hyphal networks can also function in nutrient transfers in the soil. The simplistic

source–sink transfer hypothesis has been replaced by a bidirectional translocation hypothesis sparked by studies on wood decay fungi (Connolly and Jellison, 1997; Lindahl et al., 2001). Frey et al. (2003) found that fungi transfer litter-derived C to soil macroaggregates while transferring soil-derived N to the litter layer. Carbon and nitrogen pools are altered by fungi across spatial scales and thus need to be examined at a level that allows mechanisms of plant support, soil building, and litter decomposition to be linked.

Fungal hyphal lengths correlate with a number of soil physical, chemical, and biological properties. Yet it is nearly impossible to determine the individual species or the distinct activities that result in functions such as decomposition, nutrient transfer, or host protection against pathogens or predators. Fortunately, molecular techniques are affording mycologists the opportunity to examine the species represented by hyphae, but they do not begin to provide answers to the extent or organization of fungal hyphal networks in soil. This alone would allow for the design of better and more functional sampling schemes and for understanding of the role of fungi in community and ecosystem dynamics.

Alternately, we can follow fungal spore production or appearance of fungal fruiting bodies. While we can quantify the production of spores, the temporal and spatial aspects of spore production are poorly understood. The timing and location of fungal sporulation relative to the hyphal network, nutrient supply, host, or some other stimuli are still important questions that need to be more fully addressed. Relating the appearance of spores or fruiting bodies to ecosystem dynamics also has its drawbacks because the rate of sporulation or production of fruiting bodies cannot be linked quantitatively to specific ecosystem characteristics. That sporocarps are produced indicates the presence of a belowground fungus, yet the presence of a belowground fungus is not always indicated by an aboveground sporocarp (Gardes and Bruns, 1996; Dahlberg et al., 1997). Additionally, production of sporocarps may not be related to the relative abundance of colonization of EM on roots (Clapp et al., 1995). Similar problems are encountered when characterizing the AM community based on spore counts (Bever et al., 1996, 2001). While measuring diversity or biomass may not be hampered by these results, scaling up to impacts on community structure or evaluating global climate-change effects cannot be achieved without linking spores to fungal function.

Spore counts are also difficult to evaluate because spores tend to have clumped distributions, which may cause diversity measures to change dramatically, depending on where samples are taken. The diversity of fungal spores in soil initially or following a single trapping period also may not reflect all of the species present and may be affected by the plant host. Multiple techniques are necessary to evaluate mycorrhizal species diversity under field conditions.

Studies that have examined fungi at the microscale have found patterning at this scale. Patterns of active fungal hyphal lengths were linked to vegetation patterns, topography, organic C, and moisture at small spatial scales (<1 m) (Morris, 1999). The patterns detected in microplots suggested hot spots of microbial activity that were approximately 2 cm in diameter. This was consistent with a number of other studies (Starr et al., 1992; Gonod et al., 2003), suggesting that high variation can be introduced into data sets if samples are not homogenized prior to analysis. This also means that mechanistic studies for identifying the impact of community structure and abiotic factors must be performed at the centimeter scale, whereas data for scaling up must be performed on composited samples that decrease the "noise" generated by differences in response to soil resource heterogeneity.

An additional difficulty in determining the microscale distribution of fungi in soils is the impact that they have on the microscale patterning of soils. For example, the presence of fungal hyphae has been linked to formation of water-stable macroaggregates, which is

important for protecting soil organic matter and improving soil structure (Denef et al., 2001). The mechanism by which fungi accomplish this task is obscured by poor understanding of spatial structure of bacterial vs. fungal populations, the species of fungi involved in aggregate formation, and differential impacts of wet–dry cycles on these organisms. Information on the distribution of fungi within aggregates, i.e., at the smallest spatial scales in soil, would provide valuable data on the importance of the presence of hyphae in organic matter stabilization, nutrient retention, soil stability, and soil structure. Different communities may also be involved with decomposition associated with different aggregate fractions or soil structural classifications (light fraction vs. particulate organic matter dynamics). Understanding these dynamics is essential for evaluating the roles of fungi in organic matter turnover in soils.

Microscale patterns suggest differences in fungal distribution at the millimeter scale. At the centimeter scale the distribution of fungi is also impacted by litter and soil depth. Sampling through a profile will identify different groups of organisms at different depths. Some studies have found that the degradative capacities of these organisms may not differ much from groups at other depths (Bååth and Söderström, 1980). Differences in consumption of fungal hyphae by soil organisms are also affected by the distribution of hyphae. Hyphal lengths in the litter layer are even more susceptible to faunal feeding than hyphae in lower layers (Klironomos and Kendrick, 1995b). Removal of the litter layer alters consumption patterns and density of fungal hyphae. When litter layers are removed, fungal feeders spend more time consuming mycorrhizal fungi than litter fungi. This may decrease the hyphal network of the fungus. The degree to which the extraradical hyphal network is necessary for mycorrhizal function and the length of time that it is active are currently unknown. This information is necessary to evaluate the impact of the fungal feeders on mycorrhizal functioning and NPP.

Advances using molecular techniques have also identified EM fungi distributed across different soil layers. Niche differentiation across soil substrates has been proposed to contribute to EM diversity. The research presented by Dickie et al. (2002) supports this hypothesis and detected, even with relatively shallow sampling, up to six different patterns of spatial resource partitioning from the four layers sampled (lower litter, fermentation layer, humified layer, and B horizon (2 cm below the humified layer). The results of Taylor and Bruns (1999), using molecular techniques, also identified differences in patterns among both the mature forest community and the resistant propagule community in a *Pinus muricata* forest. Their results demonstrated differences in resource preferences and colonization strategy for maintaining species richness in the EM community. These improved identification methods and microscale approaches will allow for better understanding of the distribution of fungi in soils. Ultimately, distribution patterns are influenced by more than just physiology and abiotic factors; the role of biotic patterns must also be determined. Approaches that strive to incorporate an understanding of the ecological roles that fungi play will likely result in data that will improve our understanding of fungi in ecosystems and improve our ability to design sampling schemes for studying these organisms.

Many studies have examined the scale at which other soil organisms (e.g., Robertson and Freckman, 1995) and other microbial community parameters (Arah, 1990; Boerner et al., 1996; Decker et al., 1999) exist in soils. The distributions of these organisms, which are tied to fungi through trophic or competitive interactions, contribute to the spatial distribution of fungi in soils. Studies have reported correlations between the distributions of these organisms in soil and interactions between specific groups. Specifically, increases in hyphal length are associated with fungal grazing by arthropods (Hanlon, 1981; Hedlund et al., 1991) through removal of inhibitory compounds and senesced fungal materials. Fungi have also been observed to increase arthropod fecundity (Klironomos et al., 1992). Belowground

communities are as complex as any aboveground food web but are even more difficult to study, as identity of the participants and system feedback loops are often obscured. Microscale studies are needed to examine soil food web dynamics at the scale at which they operate in intact systems or through studies that include as many of the complex interacting groups of organisms as possible in greenhouse or common garden experiments.

The assumptions currently made for individuals and their impacts at higher scales are those of linearity (Bever, 2003). Yet there is evidence of nonlinear feedback from the microbial community to the plant community. To scale from individuals to plot scale studies an understanding of the degree to which these assumptions can be made must be examined. We also need information on the degree to which the feedback mechanisms of the microbial community, both negative and positive, operate simultaneously to impact species presence in plant communities.

1.3.2 Linking from the Plot Scale to the Landscape Scale

The experimental unit for studying fungi or changes in microbial community dynamics in response to some treatment is often the plot or, in larger studies, the watershed. To adequately address research questions regarding fungi, sampling schemes at this scale must be representative of the organisms studied. Stratifying sampling schemes to include the parameters that most likely affect fungi is important. The impact of vegetation has been documented in a number of studies (Zinke, 1962; Morris, 1999), as has topography (Morris and Boerner, 1999). The latter is not surprising, as topography is often associated with moisture. Incorporating positional impacts of landscape components into sampling schemes increases the probability of decreasing random noise and improves the probability of detecting treatment differences when they exist.

Problems that may confound the ability to detect differences even after constraining for these variables are the local scale differences in fungal biomass that may not be accounted for at either local (vegetation) or regional scales. Contiguous watersheds, which are often used as treatment units, may be problematic for studying treatment impacts on fungi. One study that examined microbial community dynamics in southern Ohio found that while bacterial biomass pretreatment differed only across regions, fungal biomass differed across watersheds within a region (Morris and Boerner, 1999). The strongest predictors for fungal biomass were sand, clay, and long-term indicators of moisture patterns (e.g., slope, aspect, water-holding capacity), suggesting that fungal biomass was subject to intermediate-scale impacts that increase random noise across otherwise homogenous watersheds. Better ways to quantify fungal biomass, specifically for understanding the value of fungal hyphal lengths, are necessary to evaluate the impacts of treatments on fungi.

Fungi also differ in energy contributions to different fungal structures. Studies that examined the relative contributions of AM fungi to intraradical and extraradical hyphae, arbuscules, and hyphal coils found soil nutrient content to affect contributions to each of these structures (Johnson et al., 2003). While this approach ties structure to function, it suggests that studies that compare fungal hyphal lengths across different sites may confound locational or treatment effects by negating the contributions of small-scale differences in nutrient content on hyphal production. Treseder and Allen (2002) found changes in hyphal length following nutrient additions to be related to current site nutrient limitations and species present. These results suggest that pretreatment dynamics in fungal studies can be even more important than for other types of organisms.

Multiple studies have suggested that even at local scales fungi are elusive. Studies of a single-hectare abandoned agricultural site have yielded an unprecedented 37 species of AM fungi only after years of study using multiple approaches (Bever et al., 2001). Conventional wisdom suggests that this site should have had a low diversity of fungi directing

a high diversity of plants. These misconceptions permeate the literature and limit our ability to clarify the number of fungal species even across a single hectare. Acquiring accurate data on species numbers is essential to understanding diversity. This step must precede development of hypotheses on the mechanisms that drive diversity of these organisms.

1.3.3 Linking from the Landscape Scale to Global Scale

While differences within study areas may be difficult to detect, there has been great success identifying differences in communities as we move across landscapes. Taylor and Bruns (1999) found differences in the community structure of EM fungi in a *Pinus muricata* forest and demonstrated minimal overlap in two different groups of fungi across a disturbance gradient. Boerner et al. (1996) demonstrated differences in spatial patterns across a gradient from native systems to agricultural systems. Their results suggested that the distribution of AM propagules became more homogenous with an increase in age since disturbance. This is even more important for evaluating EM infectiveness following disturbance. Five years after disturbance, the probability of an EM-dependent seedling encountering EM inoculum was only 50%. This increased to 100% 25 to 30 years after disturbance.

Landscape patterning is important to evaluate because the distance to inoculum affects the recolonization of fungi and, thereby, plants. Recovery of vegetation following the volcanic disturbance at Mt. St. Helens was slowed by poor inoculum density and likely poor distribution of EM mating types on the most severely disturbed sites (Allen et al., 1992). Twenty years after the volcanic blast, poor development of conifers at the site is likely the consequence of the number of years spent without appropriate inoculum and the poor distribution of nutrients. Low inoculum density affected plants that associate with AM fungi less severely because they are often facultative and AM fungi have larger spores that may be more easily distributed by fossorial mammals and animals located in refugia. Evaluating the impact of landscape mosaics on distribution patterns and availability of fungi is necessary to predict recovery following disturbances, including large-scale climate change.

It is one thing to discuss the roles of fungi at each scale, but how does one approach integrating across scales to understand the overall global contribution of fungi? Plot level studies must incorporate microscale patterning in a representative way. To adequately achieve this goal, we must be able to provide information on fungal distribution patterns. We cannot currently evaluate the degree to which our methodologies are adequate to detect all of the fungi and organisms that interact with the fungi in a single gram of soil.

Mycologists have begun to identify the incremental increase in NPP contributed by mycorrhizal relationships (i.e., the contributions to aboveground and belowground food webs), but integrating the affiliated changes in plant chemistry with decomposition rates is now necessary to evaluate the impact of the mycorrhizal relationship on overall ecosystem dynamics. Additionally, changes in diversity of aboveground species will also contribute to alterations in NPP. How can this contribution be quantified if not at the individual plant scale? Mycorrhizal fungi may be a key link for understanding the tie between atmosphere and plant growth and will likely be an essential driver for evaluating the impacts of elevated CO_2 on terrestrial systems (Fitter et al., 2000). In this same way, the impacts of temperature change on decomposer fungi will likely be key to understanding feedback mechanisms in terms of nutrition and CO_2 concentrations in ecosystems.

1.4 CONCLUSIONS

Microscale patterning of soil organisms results in what is perceived as random noise when sampled at small scales (Ettema and Wardle, 2002). This is a problem for identifying the

fungal contribution to overall ecosystem function. It is essential to begin to establish the source of the noise and identify the roles of individuals and the spatial dynamics that allow them to perform these roles. Additionally, the difficulty of evaluating the composition of the microbial community and apparent simplicity with which it can be modeled has caused microorganisms of all varieties to be considered functionally redundant and, therefore, of little concern at the species or microscale level. While this may work currently for global scale modeling, failure to elucidate the contributions of individual species to the structure of communities and functioning of ecosystems will limit our ability to predict impacts of global climate change, anthropogenic disturbance, or habitat fragmentation on the resistance or resilience of ecosystems. To this end we have the following research needs:

1. To elucidate the role of specific fungi in contributions to functional processes
2. To connect hyphal networks to their function
3. To relate hyphal networks to spore counts
4. To unite DNA technologies with indices that indicate activity
5. To provide linkages across scales to other organisms in the food web

The concern of Waksman (1916) that who is active is more important than who is present has not been adequately addressed even today. However, with the advent of molecular techniques and the speed with which they have already transformed our knowledge of belowground fungal communities, we are now in a much better position to answer the challenging questions that will tie fungal community structure to ecosystem function.

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Fungal Communities: Their Diversity and Distribution

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2.1 INTRODUCTION

The word *community* has a variety of definitions, including "people having common rights," "a body of persons in the same locality," "a body of persons leading a common life," or "a monastic body" (Kirkpatrick, 1983). In ecology the term has traditionally been defined as "any naturally occurring group of different organisms inhabiting a common environment" (Abercrombie et al., 1957), but it has also been used in a much more restricted sense, for example, "a group of plants growing in a particular area, usually of distinctive character, and requiring certain physical conditions which satisfy them" (Monkhouse, 1965). The term is not featured in the standard overall microbiological glossaries (e.g., Singleton and Sainsbury, 2001), but is defined more narrowly as "any phytosociological taxon" in Kirk et al. (2001).

The concept of a group of organisms growing in a particular area and requiring similar conditions is easier to apply to some fungal habitats and groups of fungi than to others. The characterization and naming of communities and the mapping of vegetation types have been major and ongoing activities in botany that started over 80 years ago (e.g., Gams, 1918; Braun-Blanquet, 1928; Du Rietz, 1930) and have journals and series devoted to them (e.g., *Bibliographia Phytosociologica Syntaxonomica*, *Documents Phytosociologiques*, *Fitosociologia*, *Phytocoenologia, Vegetatio*), but the approach has scarcely

impacted on mycology. There are many reasons for this, but the diversity of fungi* is so great, and so much of it is as yet unknown, that a habitat or community approach has to be a surrogate for species conservation in fungi. Yet we remain far from the point where a community- or ecosystem-based system for fungi can be recommended for general use in conservation criteria or legislation at international, regional, and national levels.

The issue requires a consensual and transparent approach that could in time be adopted or endorsed by bodies such as IUCN–The World Conservation Union and the Subsidiary Body for Scientific, Technical and Technological Advice (SBSTTA) of the Convention on Biological Diversity. This vision is probably at least a decade to realization. Here we can but point to constraints, provide an eclectic view of fungal diversity across biomes and ecological niches, discuss correlations with plant and animal diversity and forces driving and influencing fungal diversity, and point to ways we might proceed.

2.2 CONSTRAINTS

2.2.1 Circumscription

A key problem in a community approach to fungal diversity and distribution is their interdependence with other organisms. With the exception of fungi that form lichens, fungi are not primary producers; in consequence, they cannot form separate self-sustaining communities, and their occurrence is irrevocably linked with that of organisms on which they depend for their nutrition. A further complication is that many fungi are found outside natural vegetation systems, occurring on particular cultivated crops or garden plants or as biodeteriogens and contaminants of foodstuffs and manufactured goods. Consequently, any attempt to circumscribe fungal communities in an independent manner, parallel to that used in botany, and without regard to the organisms on which they depend, is surely unrealistic.

2.2.2 Mycosociology

Mycosociology, the study and classification of fungal communities in their own right, has had few advocates. Höfler (1938) and Hueck (1953) considered that fungal communities could be named independently because they were dependent on factors other than those that controlled the plant communities within which they occurred, but few have endeavored to take this further. The problems have been aired by Apinis (1972) and Barkman (1973) in particular and need not be repeated here.

In the case of macromycetes, the only author to assiduously endeavor to introduce a formal system for naming fungal communities independently of the plants with which they were associated was Darimont (1975), who attempted to lay down a formal system of mycosociological nomenclature to apply to data he had collated from woodlands in Belgium, for example, using the suffixes *-ecea* (class; e.g., *Cortinario-Boletacea*), *-ecia* (order; e.g., *Boleto-Amanitecia*), *-ecion* (alliance; e.g., *Boletacion scabri*), and *-ecium* (sociomycie, equivalent to the association of phytosociologists; e.g., *Amanitecium muscariae*). He recognized 24 sociomycies in the Belgian woodlands, 18 alliances, 8 orders, and 4 classes. The approach does not appear to have been taken up subsequently.

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* The term *fungi* is used throughout this chapter to embrace all organisms that belong to the kingdom Fungi together with others traditionally studied by mycologists, i.e., lichens, slime molds, straminiples (oomycetes), and yeasts, as well as mushrooms and molds.

Some European lichenologists, however, enthusiastically adopted phytosociological principles and developed systems for the formal naming of communities regardless of the plant communities in which they occur (e.g., Barkman, 1958; Wirth, 1972). Consequently, a staggering number of Latinized community names for lichen assemblages have been proposed (Delzenne-van Haluwyn, 1976). This is not so surprising, as lichens are the predominant biomass in some ecological situations, such as on rock surfaces and on the ground in boreal and arctic-alpine situations, and those on bark can be more related to its chemical characteristics and the ambient environment (e.g., relative humidity, temperature) than the trees involved. Provided that a broad-brush approach is adopted, the use of a hierarchy of Latinized names can provide both a useful framework for the description and a shorthand method for communicating complex concepts of assemblages for lichenologists (James et al., 1977). Community names such as *Lobarion* and *Xanthorion* are consequently in widespread use in the 21st century, even though few workers now devote time to characterizing and describing lichen communities in the formal manner required by the International Code of Phytosociological Nomenclature (Barkman et al., 1986).*

The approach is unlikely to ever be fully implemented for nonlichenized fungi in view of the problems in thoroughly recording what species may be present in a particular site. However, as most other fungi are an integral functional part of plant-dominated communities (Dighton, 2003), this is surely not an inappropriate outcome of the debate as to whether fungal communities in general should be named in a formal manner. Because of issues of differing spatial scale and processes of structuring species assemblages between plants and fungi, invoking the concept of synusia (a grouping, within one layer of a community, of species characterized by similar life-forms and similar ecological requirements), or assemblage, may be of use in describing fungal species assemblages, e.g., epiphyllous ascomycetes of oak leaves, soil microfungi in particular microhabitats, and gallery beetle associate fungi.

2.2.3 Recording

The first issue to be considered in endeavoring to survey the fungi in a particular site is the number of different ecological niches in which they can occur and that have to be searched if a total inventory is to be attained. This is a major constraint in view of both the number of niches meriting scrutiny (Table 2.1) and the different expertise and methodologies required to examine the species present in many of those niches (Rossman et al., 1998; Mueller et al., 2004). The problem is then compounded by seasonality or periodicity in the production of visible fruiting bodies. In some cases fruit bodies are ephemeral and may last for only a few hours, while in others the same species may not produce fruit bodies every year, or even decade. For example, in a 21-year study of forest plots in Switzerland, Straatsma et al. (2001) encountered fruit bodies of 408 species. However, the number recorded in each year, even after repeated visits, ranged from 18 to 194, with 19 species not previously encountered at all found in the last year of study.

The time necessary to produce a definitive list requires long-term commitment and investment. The two most intensively studied sites to date are Esher Common (Surrey, U.K.) and the Slapton Ley Nature Reserve (Devon, U.K.), with 2900 and 2500 species recorded, respectively (Cannon et al., 2001). Yet while both sites have been studied for

* For a brief introduction to the complex procedures and history of the formal naming of communities in a mycological (and lichenological) context and further early references on this topic, see Hawksworth (1974).

Table 2.1 Principle Niches and Microhabitats Occupied by Fungi

Living Vascular Plants	
	Biotrophs and necrotrophs of leaves, stems, flowers, fruits, seeds, roots, etc.
	Commensals on bark and leaves (especially lichen-forming fungi)
	Endophytes of leaves, stems, bark, and roots
	Secondary colonizers of dead attached tissues and leaf spots, etc.
	Mycorrhizas (endo-, ecto-, ericoid, orchid, etc.)
	Leaf surfaces
	Nectar
	Resin
Dead Vascular Plants	
	Saprobes on wood, bark, and litter
	Burnt plant tissues
	Saprobes on submerged and inundated plants
	Pollen in water samples
Nonvascular Plants	
	Algae (marine, terrestrial, and freshwater)
	Bryophytes
Fungi	
	Biotrophs, necrotrophs, and saprobes of other fungi
	Lichenicolous fungi
	Myxomyceticolous fungi
Vertebrates	
	Skin, feathers, hair, bone, etc.
	Dung
	Nests, lairs, etc.
	Ruminant guts
	Fish scales and guts
Invertebrates	
	Biotrophs and nectrotrophs
	Arthropod exoskeletons
	Arthropod and annelid guts
	Nematodes
	Insect nests
Rock	
	Lichens
	Epilithic fungi
	Endolithic fungi
Soil	
	Surface
	Soil cores
Water	
	Foam
	Streams, permanent and temporary ponds
	Litter and wood immersed in sea- and freshwater
	Plants (e.g., bromeliads)

Adapted from Hawksworth et al. (1997) and Hyde and Hawksworth (1997).

over 25 years by numerous mycologists to reach these figures, only 40% of the species are in common despite many similarities between the localities, and additional species are added each year. The actual number of species of fungi present in these sites could easily be around 4000, but both are much disturbed and neither has a long history of ecological continuity.

In the case of the Guanacaste Conservation Area in Costa Rica, an international working group estimated that the number of fungi to be found would be around 50,000 species and that an inventory would cost U.S.$10 to 30 million over 7 years, depending on the level to which identifications were made (Rossman et al., 1998). However, even if ample funds were available, the shortage of available specialists in many groups of fungi would pose a major hurdle to such an intensive recording project.

Data quality of records is also a problem, as a significant proportion of reports of species from a particular community made by nonspecialists may be unreliable due to insufficiently critical determinations being made. Further, in many cases dried or cultured voucher material is not preserved in institutions such as herbaria, museums, and collections of fungus cultures, rendering many records of questionable long-term value (Agerer et al., 2000). Additionally, many countries do not yet have fungus recording schemes in place, and those that do are generally underfunded.

Finally, until the recent publication of recommended standardized sampling methodologies (Mueller et al., 2004), there had been little progress toward the adoption and universal use of recommended and internationally accepted standard protocols for sampling fungi in particular ecological niches, thus making comparisons of data sets from, for example, soil and leaf isolations, difficult. Similarly, suggestions to focus on target groups of fungi as surrogates for overall species richness (Hawksworth et al., 1997) have hardly progressed.

2.2.4 Diversity

The suggestion that fungi are an exceptionally diverse and poorly known group of organisms with around 1.5 million fungi on Earth, of which only 74,000 to 120,000 have so far been identified (Hawksworth, 2001), continues to be supported by fresh analyses (Heykoop et al., 2003; Mueller et al., in press). Schmit and Mueller (in press) conservatively estimate a *minimum* of 600,000 species worldwide based on the ratios of fungal-to-plant species in well-studied regions and taking into account data on endemism. This conservative figure was calculated to establish a lower boundary for the number of fungal species, which will be revised upward as more information becomes available. Whatever the final figure may prove to be, there is no doubt that the magnitude of the species numbers present in any detailed community study poses special problems, in that species that are as yet unnamed are likely to be encountered, especially when working in hitherto little-explored ecological niches or geographical locations.

Further, there is a lack of modern monographic revisions and keys for many groups of fungi, which makes the identifications necessary for community characterization particularly time-consuming.

2.2.5 Species Concepts

The species concepts traditionally used in different fungal groups vary, depending on which characteristics are considered important. However, incompatibility studies and the advent of molecular phylogenetic approaches are increasingly showing that in many cases, what has traditionally been interpreted as a single species on morphological criteria alone is in reality a complex of biologically and evolutionary distinct species. This is as true for lichen-forming fungi (Grube and Kroken, 2000) as it is for macromycetes (Petersen and Hughes,

1999) and plant pathogens (O'Donnell et al., 2000). At the extreme end of changes in a group's species richness based on molecular data are suggestions that the diversity of arbuscular mycorrhizal fungi may be closer to hundreds of thousands of species rather than the traditional view of hundreds of species (Fitter, 2003). It is clear that the formal recognition of such species will increasingly become the norm in mycology (Taylor et al., 2000). While these so-called cryptic species are evidently a major component of the estimated unrecognized global species numbers of fungi, their existence poses major problems for the documentation of fungal communities. The need to distinguish species that are so similar morphologically that they cannot be conclusively identified without cultural or molecular studies will inevitably hinder critical survey work of all kinds in mycology.

2.2.6 Fallacies

Several fallacies impinge on endeavors to characterize fungal communities, three of which are deeply embedded in the minds of many biologists:

1. "Everything is everywhere and the environment selects" (Baas-Becking, 1934). While this may be true for some saprobic, soil, and opportunistic spoilage microfungi with enormous potential for dispersal (Gams, 1992), it clearly does not apply to the huge numbers of host-specific fungi, many macrofungi (see below), or lichen-forming species. This is especially so when species are studied at the cryptic species and population levels, where species thought to have widespread distributions prove to be complexes of two or more distinct taxa (see above).
2. Most fungi occur in damp places (countries). May (1994) cautioned against extrapolations based on data from countries such as the U.K., which were "damp and fungal place[s]" from an Australian viewpoint. This may apply to some groups of fungi, but cannot be supported as a general rule as the most species-rich habitats may vary from region to region. For example, in Australia huge numbers of microfungi are found associated with native species (e.g., *Eucalyptus*; Sankaran et al., 1995), macrofungal diversity is high (T.W. May, personal communication), and the continent appears to be a gold mine for undescribed hypogeous macromycetes (Claridge et al., 2000; J. Trappe, personal communication) and certain groups of rock-inhabiting lichens (e.g., *Xanthoparmelia*; Elix et al., 1986; Elix, 2003).
3. Yeasts and lichens are not fungi. Old concepts die hard, and even to this day it is not uncommon to see phrases such as "yeasts and fungi" and "lichens and fungi," which are oxymorons.* Yeasts are firmly part of the kingdom Fungi, and strictly lichens have no names; the names used are those ruled as applying to the fungal partner (the photosynthetic symbionts maintaining independent names; Hawksworth, 1997a). It is especially surprisingly to find lichens treated in a series of floras (e.g., Flora of Australia), while the "other" fungi have an independent sister series (e.g., Fungi of Australia).

In addition, the historical inclusion of mycology within botany, and also the treatment of fungi as a kind of cryptogam or lower plant, has been deeply damaging to the perception, organization, and development of the subject (Hawksworth, 1997b).

* Oxymoron: "A figure of speech by means of which contradictory terms are combined," literally "pointedly foolish" (Kirkpatrick, 1983).

2.3 DIVERSITY ACROSS BIOMES

Fungi are so diverse that it is difficult for any single person to address the issue of diversity across biomes. However, Lodge et al. (1995) approached this by soliciting the opinions of a range of mycologists with experience in more than one continent or hemisphere. Most of those consulted who worked on basidiomycetes felt diversity was more correlated with host and habitat than resource abundance, while those whose expertise was with ascomycetes (and their hyphomycete anamorphs) considered all three factors important. The areas most frequently mentioned as sources of novel unknown taxa were humid forests on islands, tropical mountaintops, and large tropical river basins. However, some host-restricted heterobasidiomycetes occurred in all areas and habitats with the hosts, while ranges in many agarics appeared to be limited regardless of the region. Overall, diversity in most groups, except rusts and smuts, was judged to be greatest in the tropics and subtropics and most strongly related to habitat and host diversity. While this was a refreshing way to visit the overall issue, it was essentially qualitative rather than quantitative, a valuable source of hypotheses based on impressions that merit testing by substantial data sets. Mueller et al. (in press) pooled data on diversity and distribution patterns for macrofungi from different geographical regions. They compiled 21,679 names during this study and found that the percentage of unique names varied from 37% for temperate Asia to 72% for Australasia. No comparable data set for other fungal groups has been compiled and analyzed, and fresh and broader studies on the lines of those undertaken by Pirozynski and Weresub (1979) are to be commended.

A different approach was taken by Schmit et al. (2005). They undertook a metadata analysis of published and available unpublished point diversity studies of macrofungi that included species lists of the plants in the sampled plots. Macrofungal species included in the analyzed studies displayed neither larger nor smaller species ranges than the plants in the data set, and not surprisingly, the diversity of macrofungi in each site was high. While this study documented that tree diversity proved to be a good predictor of macrofungal diversity at each site, plant community data could not be used as a surrogate to predict macrofungal community composition.

Nevertheless, it appears from casual studies of the available information that some generalizations as to the major differences in the diversity of fungi and the communities developed in different biomes can be made (Table 2.2). Estimating species numbers has been attempted by extrapolation from the numbers of vascular plant species present (Rodríguez, 2000). While such approximations are open to debate, they do suggest that much of the species diversity in fungi is in the tropics and remains to be discovered (Table 2.3).

Such broad-brush approaches clearly mask differences in the diversity of fungal communities on a niche-by-niche (cf. Table 2.1) basis. For example, despite the considerable uniformity reported among soil microfungi (Gams, 1992), studies of communities in soils subject to different degrees of climatic stress suggest that the proportion of sexually reproducing species is positively correlated with increasing stress (Grishkan et al., 2003).

2.4 HOW TO PROCEED

The study of fungi as an integral part of plant communities and ecosystems, or as associates of particular animal or plant hosts and providers of essential ecological services, rather than in isolation, should be the underlying feature of future studies in fungal ecology; the approach of Dighton (2003) is commendable in this respect. The issues of diversity and

Table 2.2 Examples of Ecological Fungal Groups Predominating in Major Biomes

Biome	Fungal Groups
Arctic, Antarctic, arctic-alpine, montane, northern tundra	Lichen-forming fungi
Boreal	Lichen-forming and ectomycorrhizal fungi
Temperate	Plant-specific (especially rusts and smuts) and ectomycorrhizal fungi; also slime molds
Mediterranean and hot desert	Lichen-forming and soil fungi (including hypogeous macrofungi)
Tropical	Foliicolous fungi (including sooty molds, asterines, meliolines, fungicolous spp., foliicolous lichens), ostropalean lichen-forming fungi (including graphids and thelotremes), entomogenous fungi, endomycorrhizal fungi, and endophytic fungi

Table 2.3 Approximate Numbers of Fungi (including Slime Molds, Lichen-Forming Fungi, Straminipilous Fungi, and Yeasts) and Plants[a] (Seed Plants and Ferns) Known from Different Regions of the World

Region	Described Species	Estimated Total Species	Percentage Unknown
Asia	20,000 (70,000)	600,000 (77,000)	>95 (10)
Europe	25,000 (12,000)	65,000 (12,000)	60 (>1)
Africa	10,000 (60,000)	450,000 (67,000)	>95 (10)
North America	21,000 (18,000)	250,000 (18,000)	>90 (1)
Central and South America	10,000 (85,000)	500,000 (100,000)	>95 (15)
Oceania	6,000 (17,000)	250,000 (21,000)	>95 (20)
Antarctica	750 (2)	1,750 (2)	55 (0)
Global	72,000	1,470,000	95

[a] Plant figures are in parentheses.

Adapted from Rodríguez (2000).

complexity in communities are so immense, however, that small-scale case studies designed to test particular hypotheses are likely to be keys to the understanding of patterns and interrelationships on a global scale. Ideally, such studies should employ similar protocols in different biomes and communities across wide geographical regions, and also be integrated with more holistic ecosystem studies by broad-based plant and animal ecologists. Since the mid-1990s there has been a heightened awareness of the need for multidisciplinary approaches to ecosystem functioning, and fungal data have been taken note of in key debates and syntheses (Schulze and Mooney, 1994; Brussaard et al., 1997; Freckman et al., 1997). Regrettably, the funds to conduct large-scale new studies to address

key issues of the structure and maintenance of ecosystem function at the international level have remained elusive. In the interim, mycologists with an interest in the diversity of fungi and their roles in communities should endeavor to collaborate with one another wherever possible, in order to maximize the potential inputs that can be made to the elucidation of our understanding of fungal community ecology. The synthesis of issues and protocols by Mueller et al. (2004) should be a major stimulus to such an approach.

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3

Freshwater Fungal Communities

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3.1 INTRODUCTION

The mission of this volume, now in its third edition, has been and continues to be an attempt to integrate research on fungi with mainstream ecological concepts. It is somewhat analogous to the desire of ecologists in the 1960s and 1970s to make ecological models and predictions as precise and as accurate as those in physics (McIntosh, 1985). Ecologists were said to suffer from "physics envy"; maybe mycologists suffer from "ecology envy." This pecking order can be extended further: few mycologists study freshwater fungi, and in the second edition of this book, Suberkropp (1992) stated that "the purpose of this chapter is to examine the advantages offered by the aquatic hyphomycete community for the study of fungal ecology." The implied assumption is that research on fungi (aquatic hyphomycetes) is important primarily when it addresses topics also pursued by scientists specializing in plants or animals (other fungi), or insofar as its results can be extrapolated to other groups of organisms. While few would go that far, it is nevertheless essential that mycologists contribute to the debates on how ecosystems are organized. Fungi are key players in nature, and it cannot be assumed that results and concepts derived from plants and animals can easily be transferred to fungi.

The history of research on aquatic hyphomycetes has been reviewed elsewhere (Bärlocher, 1992a) and will be summarized only briefly. It began with Ingold's (1942) discovery of tetraradiate and sigmoid conidia in foam, which often accumulates in running waters. Ingold determined that the preferred substrates of the fungi releasing these conidia were deciduous leaves that had fallen into the stream. The first few decades following this discovery of aquatic hyphomycetes were devoted to isolating and describing more species and investigating their basic biology. The group remained relatively obscure and essentially ignored by both mycologists and stream ecologists. This changed when it was realized that leaves and needles of riparian vegetation are often the dominant suppliers of energy

to stream food webs, and that aquatic hyphomycetes are important in making these foods acceptable to stream invertebrates by a process called conditioning. Newly developed methods to estimate fungal biomass (based on ergosterol, a fungal-specific indicator molecule; reviewed by Gessner et al., 2003) and production (incorporation of ^{14}C-acetate into ergosterol; Newell and Fallon, 1991) have firmly established the significant contribution of aquatic hyphomycetes to stream communities. In leaf decomposition studies, it has become common practice, even among nonmycologists, to estimate fungal biomass by measuring ergosterol content of the decaying substrate. This approach culminated in the first estimate of total production of leaf-decaying fungi (Suberkropp, 1997). Over an annual cycle in a small, nutrient-poor woodland stream, it fell within the range of production estimates for the other two major groups of stream heterotrophs, bacteria and invertebrates.

Estimates of fungal biomass and production undoubtedly will continue to play a prominent role in stream ecology; however, several recent reviews have thoroughly discussed this topic (Gessner et al., 1997, 2003), and it will not be further considered here. Instead, I will discuss two other recent developments that have had a tremendous impact on the practice and priorities of ecological research. The first is a renewed interest in biodiversity, stimulated by the threat of accelerating extinction and the fear that this may result in the irrevocable loss of ecosystem function and services (Schulze and Mooney, 1994). The second is the introduction of highly sophisticated molecular techniques. Because of limited morphological variability, description of microbial diversity in most ecosystems has been difficult. The use of molecular characteristics (immunogenic epitopes, nucleic acid sequences) has greatly advanced the field. While the morphology of aquatic hyphomycetes is substantially more complex than that of bacteria, it is nevertheless based on a relatively small number of traits. For identification, the reproductive state has to be observed. In many cases, individual conidia are sufficient, but often their development has to be scrutinized for unequivocal results. This means that the identity of nonreproducing mycelia growing on or in leaves cannot be established by direct observation. Molecular techniques will make this possible.

The prominence of diversity/function relationships and molecular techniques in current ecological research in general is indisputable. Both will have to be addressed by scientists studying aquatic hyphomycetes as well. Conversely, results from these organisms may lead to essential modifications of the generalizations and concepts derived from other groups or ecosystems.

3.2 IDENTIFICATION OF AQUATIC HYPHOMYCETES

3.2.1 Traditional Approach

How do we determine the diversity of aquatic hyphomycetes occurring in a stream? The classical approach has been based on identifying conidia, in some cases supplemented by pure culture studies to evaluate conidial development (Marvanová and Bärlocher, 2001). This can be done by collecting substrates naturally occurring in a stream, or by exposing them in litter bags, and periodically recovering them and scrutinizing them for formation and release of conidia, which are trapped and identified. While this gives information on which species actually reproduce on which substrates, it is extremely labor intensive, especially if one wishes to provide information on relative abundances. For surveys of entire streams, a more commonly used method was introduced by Iqbal and Webster (1973): periodically, a constant volume of stream water is sucked through a membrane filter; conidia are trapped on the filter, stained, counted, and identified. If done over an entire year, this gives an integrated representation of the fungal stream community or,

more precisely, its reproductive output. Potential artifacts include the introduction of conidia from the surrounding riparian zone. The relative scarcity of aquatic hyphomycetes on substrates collected in the terrestrial habitats (Webster, 1977; Sridhar and Bärlocher, 1993) and the very pronounced peak of conidial numbers in the water column a few weeks after leaf fall (Iqbal and Webster, 1973) suggest that most conidia present in the water column are indeed formed and released in the stream.

Counting and identifying conidia on filters is tedious and time-consuming. It is therefore important to know how much water should be drawn through a filter and what fraction of a filter should be examined to characterize the conidial population carried in the water column. The only systematic study on this topic was published by Gönczöl et al. (2001). In their particular setup, scrutiny of half filters was generally satisfactory, and 1100 to 1350 ml of water had to be filtered to recover 90% of all species. It is premature to simply extend these numbers to other studies, but the issue is clearly important: more search effort will generally recover more species, leading to a species accumulation curve.

3.2.2 Molecular Approaches

Many aquatic hyphomycete species cannot always be identified from single conidia, especially if these are sigmoidal. Tradionally, a pure culture has been required, which had to be induced to sporulate. Microgenomic approaches to taxon diagnosis exploit diversity among DNA sequences to identify organisms (Hebert et al., 2003); each species is said to contain its own, unique bar code. Our lab has had some success isolating and sequencing DNA (internal transcribed spacer [ITS] region) from single spores trapped on a membrane filter (Nikolcheva, unpublished observation). This approach has proven useful to confirm identification based on morphology; it also promises to reveal which conidia belong to as yet undescribed taxa.

An even more difficult task is the identification of fungi present on decaying leaves. Direct microscopic examination reveals primarily sterile (nonreproducing) hyphae, whose identity cannot be determined. The recent development of monoclonal antibodies, to which a suitable fluorochrome is attached, has allowed the visualizing of mycelia belonging to four aquatic hyphomycete species (Bermingham et al., 1995, 1996, 1997, 2001) and the investigating of their spatial distribution on leaves. Using these antibodies in enzyme-linked immunosorbent assays (ELISAs) provided estimates of species-specific contributions to total fungal biomass on decaying leaves (Bermingham et al., 1997).

Another promising alternative to visualize the spatial arrangement of hyphae is fluorescent *in situ* hybridization (FISH). It is based on choosing a fluorescently labeled oligonucleotide probe complementary to unique species or group-specific sequences. They are designed to hybridize to target ribosomal RNA within intact cells. Two studies have achieved some success applying this technique to aquatic hyphomycetes (Baschien et al., 2001; McArthur et al., 2001).

If the main objective is an estimate of the number of different taxa on a leaf, total DNA is extracted and a chosen gene sequence amplified with fungal-specific primers. Ideally, the sequence will differ among species. By estimating the number of different sequences we get an indicator of species richness (difficulties arise when two or more species have identical sequences or when sequences vary among conspecific strains). Small differences among DNA sequences of equal length can be characterized, for example, by terminal restriction fragment length polymorphism (T-RFLP) or by denaturing gradient gel electrophoresis (DGGE) analysis. Both techniques were applied to DNA extracted from leaves decaying in streams (Nikolcheva et al., 2003). Compared with traditional techniques (identification of newly released conidia), the two molecular techniques suggested higher species richness at the earliest stages of leaf decay (7 to 14 days). This

discrepancy might be due to the presence of terrestrial phylloplane fungi, the rapid accumulation of aquatic hyphomycete conidia that fail to establish colonies, or the presence of tiny new colonies that are not yet in a reproductive phase. These possibilities can be explored further with a combination of classical and molecular methods.

An additional refinement is based on the use of primers specific for chytridiomycetes, oomycetes, and zygomycetes, three groups that have received little attention in stream ecology. Some preliminary data from our laboratory indicate that DNA from members of all groups is common on decaying leaves, though its origin (spore, hyphae) often remains unclear (Nikolcheva and Bärlocher, 2004).

3.2.3 Scaling Up

Biodiversity at a single point in space and time is not usually of much interest. Conidial dynamics over an entire year are more informative. This has usually been done by monthly or bimonthly samples over 1 year. In temperate streams, many such studies have revealed strong periodicity in the number of suspended conidia, which is clearly related to autumnal leaf fall (for review, see Bärlocher, 1992b). In addition, there are often pronounced shifts in species dominance patterns, which may be related to changing substrate availability, temperature, or a combination of the two (Chergui and Pattee, 1988; Thomas et al., 1989, 1992; Chauvet, 1991, 1992; Fabre, 1998; Gönczöl and Révay, 1999a, 1999b).

Again, it is generally impossible to capture all species; the true diversity therefore has to be estimated by statistical means. For valid comparisons of species numbers in various streams, we have to standardize sample sizes. This can be done by rarefaction: we calculate the expected number of species if all sample sizes (= number of conidia screened) were equal to the lowest one in our study (for an example, see Bärlocher and Graça, 2002). This equalizes sample size bias by increasing all biases to match the worst bias, i.e., that of the smallest sample (Rosenzweig, 1999).

Conversely, we can extrapolate from one or more samples to the actual species diversity within an area. One approach is based on an assumed species abundance distribution. Most species are rare; a few are abundant. If there is a constant ratio of abundances between neigboring species, sorted from most to least common, we have a logarithmic species abundance curve. To describe this distribution, Fisher et al. (1943) defined α, which is an index of species diversity, but it cannot be used to estimate total species number.

In many communities, the highest number of species occur at an intermediate level of abundance. If we take the log value of the number of individuals and plot it against the number of species with that abundance, we get a bell-shaped normal distribution, or a so-called lognormal distribution (Preston, 1962). This distribution has been analyzed thoroughly by May (1975), based on the claim that it was among the best confirmed patterns in nature. Many theoretical ecologists are no longer convinced that this is the case (Rosenzweig, 1999). Nevertheless, the lognormal distribution has recently been applied to estimate bacterial diversity. If the total number of individuals (N_T) and the number of the most abundant species (N_{max}) can be determined, we can estimate the total number of species (Curtis et al., 2002). In the case of bacteria, the total abundance could be estimated by epifluorescence microscopy, and the most abundant species by FISH. Or, the two numbers could be based on relative abundance of the most common sequence in a clone library. Currently, there are practical problems with both approaches: FISH assays have been done for only a few species, and it is not known which are likely to be most abundant. Clone libraries are biased by the initial polymerase chain reaction (PCR) and are rarely sequenced to exhaustion (Ward, 2002).

If aquatic hyphomycetes obey a lognormal distribution, the approach by Curtis et al. (2002) could be extremely useful: we could simply determine the ratio between the most common type of conidium and the total number and estimate total species number. I applied

Table 3.1 Analysis of Aquatic Hyphomycete Communities in Five Streams

Site	Samples	Fit	N_t/N_{max}	S_{est}	S_{obs}	Reference
Catamaran Brook	12	IV	1.79	7	47	1
	12	II	4.49	194	46	1
	12	IV	2.28	10	46	1
	12	I	2.50	13	49	1
	12	III	2.84	19	56	1
	60	III	2.85	20	75	1
Csömöle	6	IV	2.07	8	34	2
Morgó, Site III	6	I	8.91	$3*10^5$	50	3
Coweeto	11	III	3.58	50	26	4
Sandy	11	I	1.49	7	12	5

Note: Samples: Number of sampling occasions, generally at monthly intervals extending over a year. In Catamaran Brook (Bridge site), 60 consecutive monthly samples were collected. They were analyzed separately for each year and combined over all 5 years. Fit: Maximum likelihood analysis of data fit to lognormal distribution by Regress$^+$: I, acceptable; II, marginally acceptable; III, unacceptable; IV, very unacceptable (McLaughlin, 1998). N_t/N_{max}: Ratio of most common conidia to total conidia. S_{est}: Species number estimated as described by Curtis et al. (2002). S_{obs}: Observed number of species. References: 1, Bärlocher (2000); 2, Gönczöl and Révay (1999a); 3, Gönczöl et al. (1999); 4, Gulis and Suberkropp (2003); 5, Suberkropp and Chauvet (1995).

this method to data from five studies, where conidia were sampled during all seasons (at least six samples over a year). One study (Bärlocher, 2000) extended over 5 years; these data were evaluated separately for each year, as well as in their entirety. The data were tested for lognormality with Regress+ (maximum likelihood estimates) (McLaughlin, 1998). Unfortunately, the fit was acceptable or marginally acceptable in only four of the nine 1-year data, and unacceptable for the 5-year data (Table 3.1). Not surprisingly, the test performed poorly when estimating the number of species: in 7 of 10 cases, it was below the number actually found; in one case, it exceeded the total number of aquatic hyphomycete species that have been described to date by a factor of approximately 6000.

More conventional approaches to estimating total species number from one or more samples have been discussed by Colwell and Coddington (1994), Rosenzweig (1999), and Krebs (1999). If a single sample is available, Chao's (1984) estimator often performs reasonably well:

$$S_T = S_{obs} + \frac{a^2}{2b} \quad (3.1)$$

where S_T = estimated total number of species, S_{obs} = observed number of species, a = number of species represented by one individual in the sample, and b = number of species represented by two individuals in sample.

3.2.3.1 Temporal Variability

When several samples are available, the total number of species is assumed to correspond to the asymptote of the species accumulation curve. Any number of curves can be used; frequently, a rectangular hyperbola (more familiar as the Michaelis–Menten equation used

Table 3.2 Comparison of Observed and Estimated Numbers of Fungal Species in Catamaran Brook

	Bridge	Mouth
Observed	75	70
ACE	78	71
ICE	89	83
Chao1	78	70
Chao2	92	80
Jack1	91	84
Jack2	99	89
Bootstrap	82	77
MMRuns	84	81
MMMeans	84	81

Note: Conidia were counted and identified during five successive years at two sites (Bridge and Mouth; Bärlocher, 2000). Computations were run with EstimateS (Colwell, 1997). ACE, abundance-based coverage estimator; ICE, incidence-based coverage estimator; Chao1, Chao (1984); Chao 2, Chao (1987); Jack1 and Jack2, Jackknife estimator, based on resampling without replacement; Bootstrap, based on resampling with replacement; MMRuns and MMMeans, based on Michaelis–Menten asymptote.

in enzyme kinetics) has been fitted to the data. This often gives a reasonable fit, even though it does not go through the data pair (1,1) (Rosenzweig, 1999; a sample size of one should have a species number of one). Other estimators have been adapted from capture–recapture studies (Chao, 1987; Colwell and Coddington, 1994; Krebs, 1999; Rosenzweig, 1999). I applied a number of these estimators to the data from a stream in New Brunswick (Bärlocher, 2000). They suggest that exhaustive sampling would have increased the total number of species found in this stream by 0.5 to 31% (Table 3.2). More importantly, the data show that a 1-year survey of a stream, with monthly samples, may reveal only between 41 and 75% of the species found in a 5-year survey. Have these "missing" species simply dropped below the level of detection, or have they temporarily and locally become extinct? Many of them do indeed occur at very low frequencies (some are represented by a single conidium in 60 samples), but even the more common species fluctuate considerably from year to year (see also Mothe-Jean-Louis, 1997). The ranks of the top 7 (10) species at the Bridge (Mouth) site did not differ significantly from each other, suggesting that their occurrence or relative dominance is not regulated by strict and constant niche differentiation. This is further supported by the finding that, without exception, all significant correlations between conidial abundances of various species were positive. Identities of these species, however, were not constant from year to year. These observations are compatible with the concept of scramble or exploitation competition (Hutchinson, 1978). The supply of leaves is strongly seasonal. Once they become available, many aquatic hyphomycete species scramble to colonize the empty substrates. In any 1-year period in the Bridge section, there was space for between 46 and 56 species, which

was not consistently occupied by the same species. Only 30 species occurred in all 1-year periods. Extensive intermingling of mycelia on leaves (Bärlocher and Kendrick, 1974; Shearer and Lane, 1983; Chamier et al., 1984) further suggests that active interference among species is rare, and that there is considerable functional redundancy.

3.2.3.2 Spatial Variability

Sampling in the Catamaran Brook study was restricted to two fixed sites. Based on the average downstream displacement of suspended conidia, collecting at one site would capture conidia produced within an upstream reach of a few hundred meters (Thomas et al., 1991a, 1991b; Bärlocher, 1992c). This is clearly inadequate to represent spatial variability. The species area equation (Equation 3.2) describes the number of species encountered as the search area is expanded (S = number of species, A = area, C and Z = constants).

$$S = C \cdot A^{Z} \tag{3.2}$$

This approach is appropriate if one wishes to describe large-scale geographic patterns with explicitly heterogeneous areas, or gradients of space (Colwell and Coddington, 1994). Sampling over gradients of time is logically similar; this might include sampling over a successional period or sampling over evolutionary periods.

When an increasing number of streams within a region are sampled, the number of species does indeed increase (Bärlocher and Rosset, 1981; Bärlocher, 1987; Gönczöl and Révay, 1992; Fabre, 1996). For example, Wood-Eggenschwiler and Bärlocher (1983) sampled four streams each in four regions. Within any one stream, there were between 23 and 42 species; the number for all 16 streams was 50. If we assume that each collection site corresponds to the same area (for example, a riparian corridor of a certain length and width), we could use such data to determine Z (slope or curvature of the species area curve; Equation 3.2) and estimate the total number of aquatic hyphomycete species of a larger area (Krebs, 1999). Using the numbers of Wood-Eggenschwiler and Bärlocher (1983), Z could be as low as 0.06 or as high as 0.27. The normal range for islands is 0.20 to 0.35; for nonisolated areas on an island or mainland, it falls to 0.12 to 0.17. It is also generally lower for microorganisms (e.g., 0.043 for ciliates vs. 0.31 for insects; Finlay, 2002). Lower values imply a shallow species accumulation curve. Streams can be interpreted as islands, but aquatic hyphomycetes do occur in terrestrial habitats as well as in groundwaters (Bärlocher, 1992b; Krauss et al., 2003b), which probably facilitates movement by fungi from stream to stream.

Not surprisingly, many species are essentially ubiquitous, others may be restricted to temperate or tropical regions, and only a small minority are restricted to a very small area (Wood-Eggenschwiler and Bärlocher, 1985; Marvanová, 1997). This makes it difficult to use local–regional richness plots, an approach that has become popular for characterizing the fundamental limits to species diversity (Terborgh and Faaborg, 1980; Srivastava, 1999). Broadly speaking, local diversity can be limited by ecological or evolutionary causes. Species may be locally excluded by upper limits to niche packing and minimum viable population sizes. Increasing the regional species pool would have a negligible effect on local diversity because the community is saturated. The species richness of unsaturated local communities, on the other hand, tracks that of the regional pool. When plotting local diversity (dependent variable) vs. regional diversity (dependent variable), a linear correlation suggests evolutionary constraints, whereas a curvilinear (asymptotic) relationship suggests that local diversity is limited by ecological constraints. One of the difficulties lies in identifying local and regional scales for microorganisms: local implies a scale small

enough that all species could encounter each other within ecological time (in aquatic hyphomycetes, this could correspond to a stream or to a stream section). The regional species pool contains all species that could potentially colonize a location if competitive exclusion were unimportant.

Given the apparently worldwide distribution of many aquatic hyphomycetes, is it possible to distinguish different regions (except temperate/cold vs. tropical)? Organisms <1 mm in size are small enough to be dispersed passively in the atmosphere and sufficiently abundant that populations can be relatively homogeneous on a global scale (May, 1990). "Bugs are everywhere" (Godfray and Lawton, 2001): this has recently been shown for ciliates and flagellates (Finlay and Clarke, 1999) and has been suggested for aquatic hyphomycetes (Wood-Eggenschwiler and Bärlocher, 1985). The ratio of species occurring in one stream (Catamaran Brook; Bärlocher, 2000) to the total number of described species is approximately 1:3, which exceeds, by several orders of magnitudes, the ratio for metazoans (e.g., Fenchel, 1993).

To contrast spatial to temporal variability, it would be interesting to follow the fungal populations in several streams over several successive years: Are the dynamics of a given species synchronized among neighboring streams? Or, are there subpopulations, whose spatial extensions change largely independently of each other? Again, molecular techniques will be indispensible in determining the degree of connectedness among these subpopulations (Laitung, 2002).

3.2.3.3 Factors Controlling Local Species Diversity

The impetus for the surveys cited above was a search for factors that affect the diversity of aquatic hyphomycetes. Initially, the emphasis was on a comparison of water chemistry (pH, alkalinity, Ca^{2+}) vs. riparian vegetation. Early studies suggested high fungal diversity over a relatively broad pH range, with values below 4.5 to 5.0 and above 7.5 to 8.0 (Bärlocher and Rosset, 1981; Wood-Eggenschwiler and Bärlocher, 1983; Bärlocher, 1987). Later studies gave ambiguous results (Bärlocher and Petersen, 1988; Chauvet, 1992; Gönczöl and Révay, 1992; Raviraja et al., 1998), and Chamier (1992) concluded that pH may have an indirect effect (e.g., by affecting the solubility of Al or other metals).

Wood-Eggenschwiler and Bärlocher (1983) observed no correlation between four broadly defined vegetation types and fungal species richness. More detailed studies found a positive correlation between fungal diversity and the diversity of riparian vegetation (Fabre, 1996), or substrate diversity in the stream (Laitung, 2002), though the connection is not universal (e.g., Gönczöl et al., 1999).

There is little doubt, however, that some leaf species consistently acquire more fungal species more rapidly; in temperate streams, alder leaves are among the best baits for aquatic hyphomycetes, while conifer needles are among the worst (Bärlocher, 1992c). Colonization of leaves of *Eucalyptus globulus*, widely planted to produce pulp and paper, is delayed due to cuticle and associated waxes and to high levels of inhibitory substances (Bärlocher et al., 1995; Chauvet et al., 1997; Canhoto and Graça, 1999). Not surprisingly, replacement of mixed deciduous forests by eucalypt monocultures depressed fungal diversity in nearby streams by approximately 28%, which nevertheless is far from a linear response to the loss of riparian plant or stream detritus diversity (Bärlocher and Graça, 2002).

Fabre (1996, 1998) concluded that fungal and riparian tree communities are simultaneously but independently structured by environmental conditions, summarized by elevation. The effects of altitude and longitudinal changes along a water course have also been noted by Shearer and Webster (1985), Chauvet (1991, 1992), Raviraja et al. (1998), and Gönczöl et al. (1999).

A potentially important factor, ignored until recently, is the supply of inorganic nutrients (Suberkropp and Chauvet, 1995): in four Alabama streams with very low nitrate-N levels (7 to 24 $\mu g\ l^{-1}$), an average of five species were found. The number was twice as high in six nearby streams with generally higher nitrate levels (18 to 298 $\mu g\ l^{-1}$; all streams in this group also had higher phosphate-P levels). Interestingly, a recent report suggests that human activities have substantially increased the level of inorganic nitrogen in natural waters. In 100 streams running through unpolluted, primary forest in temperate South America, inorganic nitrogen ranged from 0.02 to 7.7 μg N l^{-1} vs. 350 to 766 μg N l^{-1} in five old-growth forests in North American catchments (Perakis and Hedin, 2002). It would be extremely interesting to determine fungal diversity in these remote South American streams; based on current evidence, I would suspect it to be very low, maybe even lower than in the Alabama streams studied by Suberkropp and Chauvet (1995). If confirmed, human activity may in fact have increased local diversity of aquatic hyphomycetes — at least in moderately polluted streams. Equally interesting would be comparisons of species accumulation curves in pristine and moderately polluted streams. Do they differ in Z (curvature of species accumulation) or C (similar curvature, but different starting points) or both? Addition of N and P tends to increase growth, reproduction, and decompositional activities of aquatic hyphomycetes (Suberkropp and Chauvet, 1995; Chauvet et al., 1997; Sridhar and Bärlocher, 2000; Gulis and Suberkropp, 2003). This may result in larger, less fragmented fungal populations. Local occurrence of species thus stimulated would therefore be less sporadic, which could lead to increased species diversity. This topic is discussed further below.

3.2.3.4 Substrate Specificity

It is generally believed that most aquatic hyphomycete species can colonize and grow on a wide range of substrates (Webster and Descals, 1981; Bärlocher, 1992c; Suberkropp, 1992). Nevertheless, the relative frequencies of individual species are influenced by the substrate. For example, dominate species on conifer needles differ from those on deciduous leaves (Bärlocher, 1982), and fungal communities of streams running through eucalypt stands are more similar to each other than to those running through mixed deciduous forest (Bärlocher and Graça, 2002). Such differences are particularly pronounced when leaves are compared with wood or grasses (Gulis, 2001).

3.2.3.5 Infraspecific Genetic Variation

Infraspecific genetic variation is a component of diversity that has only recently become accessible through molecular techniques. Three studies have been published addressing this topic in an ecological context. Peláez et al. (1996) determined random amplified polymorphic DNA (RAPD) profiles of the two dominant species, *Heliscus lugdunensis* and *Articulospora tetracladia*, in a Spanish stream. Over 100 strains of both species were isolated from foam accumulations spread over a stream reach of approximately 1 km. They could be subdivided into 5 and 7 RAPD types, respectively. The distribution of these types among foam samples appeared random and provided no evidence that there was an orderly succession of the various genotypes along the stream section.

Charcosset and Gardes (1999) applied RAPD analysis to 96 strains of *Tetrachaetum elegans*, isolated from single conidia harvested from seven species of plant leaves. Thirteen RAPD types were delineated. Typically, several genotypes were isolated from a single leaf, suggesting multiple colonization by genetically distinct spores. Numbers of genotypes per leaf species were not significantly different (χ^2 test, $p = 0.23$). Nevertheless, some

genotypes were abundant on leaves of certain plant genera and rare or absent on others, which indicates a degree of substrate preference by the various strains of *T. elegans*.

The same species was analyzed with the generally more sensitive amplified fragment length polymorphism (AFLP) technique (Laitung, 2002). A total of 97 strains were isolated from single conidia, collected from nine different streams and four leaf species. Stream location accounted for 20% and substrate for 5% of molecular variance of 32 AFLP loci (analysis of molecular variance; Laitung, 2002).

Taken together, these results suggest relatively little differentiation among conspecific strains of aquatic hyphomycetes, at least within a geographically limited area.

3.3 DIVERSITY AND ECOLOGICAL FUNCTIONS

Much of the recent interest in biodiversity was stimulated by the concern of accelerating species extinction, which may ultimately jeopardize essential ecological functions (Schulze and Mooney, 1994). This topic has produced an enormous amount of experimental and theoretical research (for reviews, see Gardner et al., 2001; Kinzig et al., 2002; Loreau et al., 2002; Wardle, 2002). Much of the early work was done on terrestrial plant diversity and its connection with production or biomass accumulation, and generally demonstrated an asymptotic relationship between diversity and function. However, both magnitude and direction of diversity effects are highly context specific (Cardinale et al., 2000; Klironomos et al., 2000; Jonsson et al., 2001). In particular, adding nutrients to an ecosystem generally lowers plant species numbers while raising productivity (Huston, 1994), implying that both richness and productivity are determined by nutrient availability. It often results in a humpbacked relationship between the two in natural ecosystems, meaning that diversity is low when resources are very scarce or superabundant (Huston, 1994; Waide et al., 1999). This applies to a local level; at regional scales, diversity tends to increase monotonically with productivity (Waide et al., 1999; Godfray and Lawton, 2001).

Aquatic hyphomycetes decompose plant remains (Bärlocher, 1992a; Suberkropp, 1992; Gessner et al., 1997). There are numerous aspects to this function (see below), but essentially it affects autotrophs (by liberating minerals), invertebrates (by conditioning of plant litter), and other microorganisms (by competing for resources, or potentially by complementary interactions). Few studies have addressed effects of fungal diversity on these various roles.

3.3.1 Field Studies

Heavy metals are released into the environment primarily through anthropogenic processes. This type of pollution generally lowers biodiversity in terrestrial and aquatic ecosystems (Lovely, 2000; Prassad, 2001). Even essential metals are toxic at high levels; nonessential heavy metals cause damage at low concentrations (Wainwright and Gadd, 1997). Growth of aquatic hyphomycetes is generally less sensitive to metal exposure than conidium production (Abel and Bärlocher, 1984; Miersch et al., 1997; Niyogi et al., 2002b, 2002c). Reduced reproduction and growth push some species below the replacement level. Fungal diversity and fungal functions (production, litter decomposition) in polluted streams generally decline in parallel (Bermingham, 1996; Sridhar et al., 2002; Krauss et al., 2003a). There are exceptions: increased Zn concentrations or low pH in streams affected by mine drainage lowered diversity but had little effect on fungal biomass or respiration (Niyogi et al., 2002b, 2002c). This may have been due to compensatory growth of the remaining species or to release from invertebrate feeding pressure. Niyogi et al. (2002a) suggest that diversity is more sensitive to low stress levels than community

biomass or function. Under high-stress conditions, the overall function of the few surviving species declines as well.

A similar argument can be made for other physicochemical parameters (Pattee and Chergui, 1995). In particular, oxygen supply is crucial for aquatic hyphomycetes. In river networks, fungal diversity was highest in the main channel (oxygen saturation, rapid decomposition of leaves), lower in side arms (intermediate decay rates, sluggish current), and practically absent in an oxbow lake (decomposition incomplete, anoxic sediments) (Chergui and Pattee, 1988).

The addition of inorganic nutrients, especially N and P, often stimulates fungal growth, reproduction, and decompositional activity (Suberkropp and Chauvet, 1995; Chauvet et al., 1997; Sridhar and Bärlocher, 2000; Bärlocher and Corkum, 2003; Gulis and Suberkropp, 2003). This may allow more species to survive and actively participate in decomposition within a stream or stream reach, resulting in a positive relationship between diversity and function — at least if one moves from very low to elevated nutrient concentrations. However, diversity by itself is not necessarily the decisive factor: Portuguese streams bordered by deciduous forest had an average of 50 species vs. 36 in streams bordered by eucalypt plantations (Bärlocher and Graça, 2002). Decomposition rates in the two groups of streams were indistinguishable; however, in comparable Spanish streams they responded to the level of dissolved N and P (Chauvet et al., 1997).

At higher levels, fungal diversity and function may be inversely related. For example, spore production was higher in hard water streams than in soft water streams, while the opposite was true for species richness (Wood-Eggenschwiler and Bärlocher, 1983; Gönczöl and Révay, 2003a). In an Indian stream, organic pollution severely depressed the diversity of aquatic hyphomycetes but did not have an equally strong effect on ecological functions that are generally associated with this group (Raviraja et al., 1998); a similar effect was found with invertebrates in a Portuguese stream (Pascoal et al., 2001). With increasing nutrient levels, fungal populations presumably grow faster. They will reach a state of intense interspecific competition more quickly, which may limit the number of coexisting species (for a review of potential mechanisms, see Rajaniemi, 2003). Combined with low diversity when nutrients are scarce, this may lead to the hump-shaped connection between productivity and diversity familiar from higher plants. Even without species loss due to competition, the number of surviving fungal species is bound to decline at very high nutrient levels due to osmotic stress (Sridhar and Bärlocher, 1997) — as will fungal metabolism and thus contribution to ecological processes. Therefore, both the number of species and the intensity of their ecological function (growth, decomposition, reproduction) will be low at very low or very high levels (Figure 3.1). If we now plot species number as X (generally reserved for the independent variable) and function as Y (generally the dependent variable), we get a total of nine relationships (three of them identical), depending on whether ecological function or species number peak at a lower nutrient level (based on current knowledge, the latter seems more likely). They illustrate the type of diversity/function relationship we might encounter if we determined the number of aquatic hyphomycete species and decomposition rates in various streams — clearly different from the asymptotic curve generally found in experimental studies (Kinzig et al., 2002; Loreau et al., 2002; Wardle, 2002).

3.3.2 Experimental Studies

An asymptotic function/diversity relationship has commonly been found in studies measuring an ecological function (production, decomposition) in microcosms or plots seeded with an increasing number of species. This diversity effect has generally been greatest at low species numbers (up to 10). Its relevance may be most easily recognized if one starts

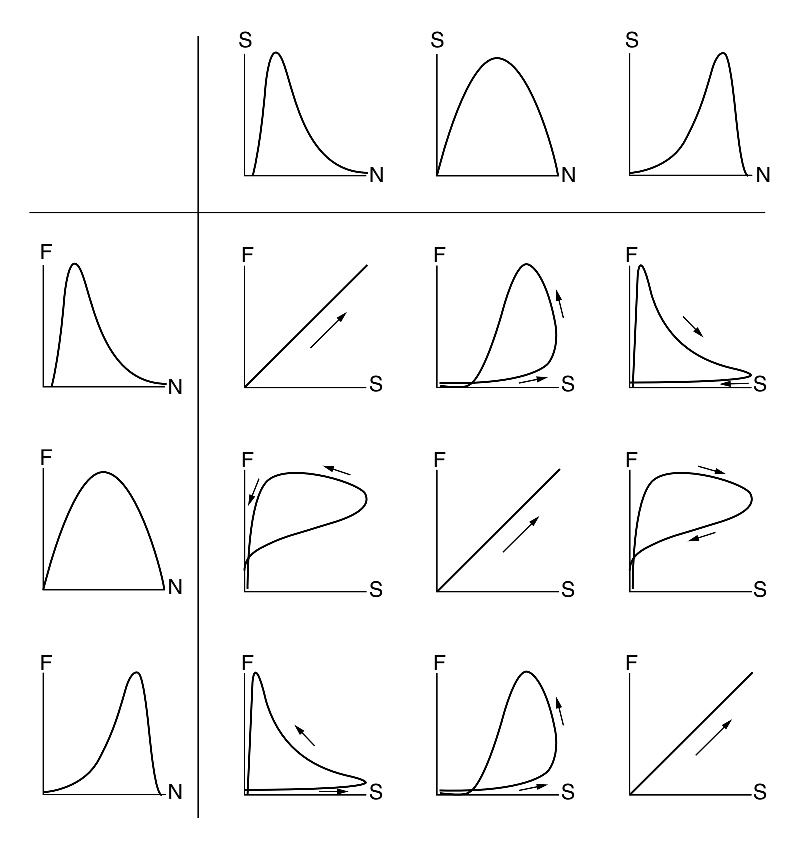


Figure 3.1 Top row: Proposed relationships between nutrient level (N) and number of fungal species (S). Left column: Proposed relationships between nutrient level (N) and ecological function, e.g., decomposition (F). Combining the two gives nine potential relationships (three identical) between species number and ecological function. Arrows indicate increasing N levels.

from the opposite end (Figure 3.2). Assume we have a certain combination of substrate, external nutrients, and a pool of fungal species, and we measure a chosen ecological function. Now assume one or more species become extinct. How will this affect the combined contribution of the remaining species? A majority of ecologists would expect a decline; Wardle (2002) believes this conclusion is premature, especially where soil organisms are concerned. Depending on species identities, any of the four possibilites in Figure 3.2 may be observed.

If function does increase with diversity, it may be due to a sampling effect; the increased probability of encountering unusually productive species increases with diversity (Aarsen, 1997; Huston, 1997; Tilman et al., 1997). If more productive species are also more competitive and can displace others, an asymptotic relationship between diversity and function will result, with the upper limit in mixed cultures defined by the top-performing monoculture (Tilman and Lehman, 2002). If complementary or facilitation interactions dominate, ecological function will increase monotonously with diversity and multicultures will outperform the top monoculture (niche differentiation models; Tilman and Lehman, 2002). The one published study on aquatic hyphomycetes with one to five species reported significantly greater mass loss in mixed cultures than predicted from average contributions of the component species in single cultures (Bärlocher and Corkum, 2003). This may have been due to sampling effects or complementarity. But the magnitude

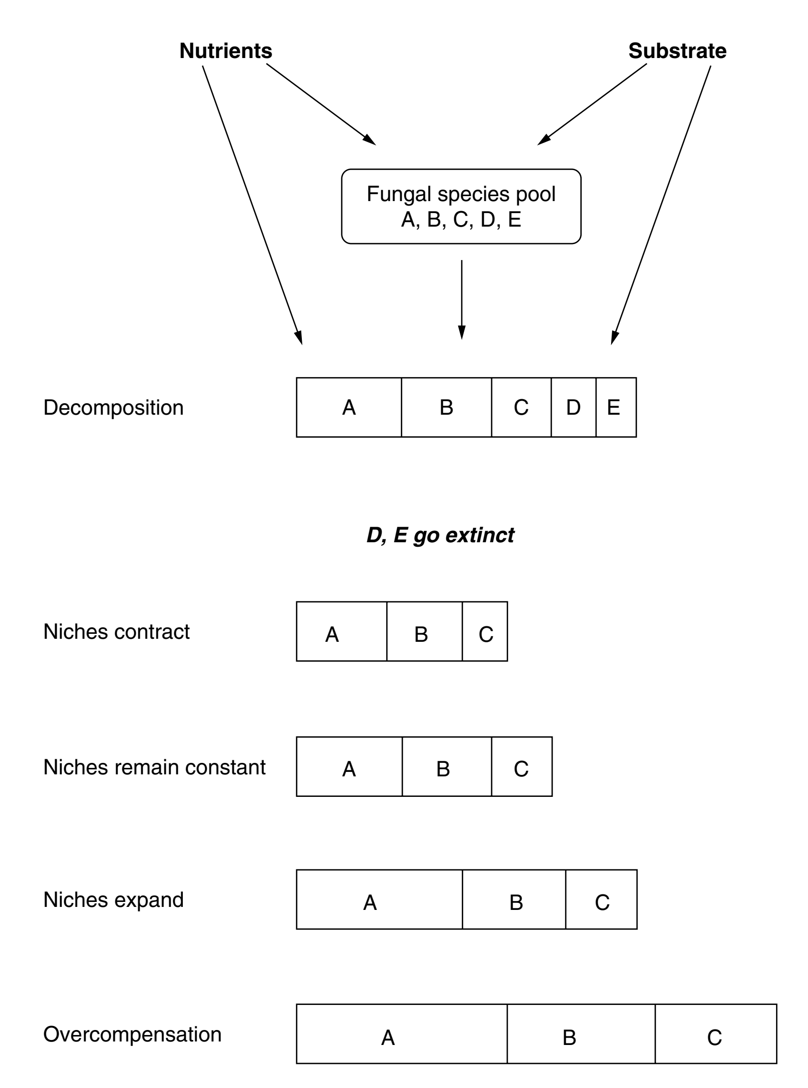


Figure 3.2 External nutrient levels and substrate surface and chemistry select which fungi from a species pool colonize. The identities of successful colonizers, substrate composition, and nutrient levels interact to determine decomposition rates. If some species disappear from the species pool due to extinction, the niches of the remaining species may contract, remain constant, or expand, resulting in lower, identical, or higher decomposition rates.

of the diversity effect was considerably smaller than that of nutrient enrichment. Combined with other evidence, the (preliminary) conclusions are that (1) there is considerable functional redundancy among aquatic hyphomycete species; (2) diversity effects are small, restricted to species-poor communities; and (3) nutrients and maybe other factors are more important.

3.3.3 Refining Ecological Functions: Münchhausen's Statistical Grid

Decomposition (or primary production) is a very broadly defined function and can easily be subdivided. For example, mass loss may be influenced by the combined activities of cellulases, hemicellulases, pectinases, and ligninases. In addition, we might measure CO_2

evolution, the release of dissolved organic substances, colloidal substances, and fine particulate organic matter, or any number of related processes (Gessner et al., 1997). If we simultaneously consider 10 such subfunctions at various diversity levels, there is a 50% chance that at least one of them will give us a *p* value of 0.05 in the absence of a real effect. This artifact of multiple comparisons has been labeled Münchhausen's statistical grid (Graham Martin, quoted in Westfall and Young, 1993). In a medical context, it allows rescuing a new, ineffective drug by subdividing patients into categories (gender, age, profession). Conversely, it can also make it essentially impossible to demonstrate the safety of any new drug or food. One simply has to expand the list of potentially harmful side effects ("casting the net more widely"). Applying this to aquatic hyphomycetes, it seems a foregone conclusion that no two species share an identical niche; the loss of any species will, therefore, represent a loss of some function. Overemphasizing such small differences, however, may divert from a more holistic point of view and deteriorate into semantics (for some examples, see McIntosh, 1985). In addition to determining levels of significance, estimating effect sizes is crucial. It is nevertheless essential to keep in mind that identical mass loss rates, performed by different combinations of fungal species, may leave behind substrates of substantially different compositions, which may affect leaf-eating invertebrates.

3.4 OUTLOOK

Major goals arising from the concern about accelerating extinction rates have been accurate identification and an estimate of total numbers of species. Hebert et al. (2003) argue convincingly that these tasks cannot realistically be accomplished by traditional, morphology-based taxonomy. Instead, they suggest using DNA sequences as bar codes. For animals, cytochrome c oxidase I may be useful as a global bioidentification system (among its advantages are haploid inheritance, limited recombination, and no introns). The same gene has been used for fungi (Cummings et al., 1989) and oomycetes (Martin and Tooley, 2003), though large and small subunit rRNA genes have generally been preferred (Bruns et al., 1991). Combined with other techniques such as T-RFLP or DGGE, this approach allows estimating the total number of different fungal strains in an environmental sample, such as a leaf decaying in a stream (Nikolcheva and Bärlocher, 2002; Nikolcheva et al., 2003). By itself, this information does not establish how many of these are aquatic hyphomycetes. This ecological group is polyphyletic, and there are no known primers amplifying exclusively DNA sequences of its members. Similarly, estimates of the total number of species of aquatic hyphomycetes cannot be achieved solely by molecular techniques.

Habitat destruction is an important cause of local extinction for all species. Targeted hunting or harvesting presents additional hazards for larger animals and plants but is largely irrelevant for microorganisms. For the latter, local physicochemical conditions, food supply, and biological interactions determine whether a given species is able to maintain itself.

Local conditions may deteriorate and cause extinction of a species. What if they subsequently improve again? Large organism may fail to reappear if extinction has occurred over large regions. It has been argued (Finlay et al., 1997; Finlay and Clarke, 1999; Finlay, 2002) that for eukaryotic microorganisms, recolonization is much more likely and may approach certainty for the following reasons: (1) few microbial species have biogeographies (they occur worldwide, resulting in flat species area curves), and (2) they are so abundant that continuous large-scale dispersal sustains their global distribution. It follows that the species richness of microbial seed banks of any ecosystem is so abundant

that microbially mediated ecosystem function will never be compromised by lack of microbial diversity. Finlay et al. (1997) assume that all vacant microbial niches are quickly filled and that the number of microbial species active at any point in time depends on the number of microbial niches available. Microbial activity and diversity, therefore, cannot be separated from ecosystem function, and it is extremely unlikely that microbial diversity will ever be reduced to the extent that the microbial community cannot play its full part. These arguments are based on work with flagellates, ciliates, and diatoms, but undoubtedly many of the same facts appply to aquatic hyphomycetes. There may be some important modifications, however. Aquatic hyphomycetes, at least in temperate regions, experience very pronounced boom–bust cycles in connection with the autumnal leaf fall. The buildup of fungal populations in fall appears to be initiated by very small numbers of conidia. This introduces an element of chance into community structures. Even the most abundant species is unlikely to be omnipresent; displacement of inferior competitors is therefore likely to be delayed. This may explain the observation that the number of species observed in any 12-month period is relatively constant, but only 40% consistently occurred in five successive periods (Bärlocher, 2000).

The addition of nutrients may speed up the elimination of competitively inferior species, at least locally (Caswell, 1978; Huston, 1979), and pollution may empty an entire stream of aquatic hyphomycetes. How long would it take for a stream emptied of aquatic hyphomycetes to reacquire a full complement of species: years, decades, or centuries? Essentially nothing is known about the long-distance transport of aquatic hyphomycetes; it has been suggested that meiospores, hyphae embedded in substrates, and conidia may be involved (Bärlocher, 1992b). In addition, aquatic hyphomycetes occur at low densities in terrestrial habitats, in roots of riparian trees, and even in tree holes (Gönczöl and Révay, 2003b). These reservoirs may facilitate recolonization of impoverished streams.

Finlay and Clarke (1999) believe it unlikely that microbial morphospecies harbor large numbers of physiological species; nevertheless, aquatic hyphomycete species have been shown to contain strains that show some stream or substrate preferences, pointing to a degree of endemism (Charcosset and Gardes, 1999; Laitung, 2002).

Finlay et al. (1997) restrict their arguments to purely microbial systems; aquatic hyphomycetes (and most microorganisms) affect invertebrates and other organisms. At least in laboratory experiments, invertebrates selectively feed on some fungal species and reject others (Bärlocher and Kendrick, 1973; Arsuffi and Suberkropp, 1989; Rong et al., 1995). Would declining fungal diversity on decaying leaves have any effect on leaf-feeding invertebrates? Or would the factor causing fungal decline have a greater direct effect on invertebrates (e.g., Cd contamination of stream water depresses fungal activity and diversity and, therefore, leaf conditioning but kills invertebrates through their intake of contaminated water rather than food; Abel and Bärlocher, 1988).

Nitrogen and phosphorus generally stimulate mass loss of leaves through increased activities of aquatic hyphomycetes. On the other hand, nitrogen can depress degradation of wood: *in vitro*, some white-rot wood decay fungi produced ligninolytic enzymes only when limited by N (Keyser et al., 1978; Kirk, 1987). Changing nutrient regimes may thus affect the balance between aquatic hyphomycetes and fungi dominating wood decay. In soils, lignin decomposers are primarily agaric basidiomycetes and xylariaceous ascomycetes. In streams, a large number of ascomycetes have been described from woody debris (Shearer, 1993), which is also, to a lesser extent, colonized by aquatic hyphomycetes and may serve as a reservoir for recolonization of leaves (Shearer, 1992).

Spatial and temporal variability of aquatic hyphomycetes, their individual responses to pollution and nutrients, and their interactions with invertebrates and other fungi make investigations of their diversity/function relationships highly complex. It is clear that even

the most diligent research group will be able to test only a minuscule fraction of all potential combinations of factors. Similarly, Maurer (2003) states that "there is a lurking sense that a single evaluation of all potential causal factors underlying how biodiversity might affect ecosystem function is beyond current (or even conceivable) theoretical tools." I feel that a useful approach may be to proceed from short-term, small-scale experiments, corresponding to small patches of leaves in a stream, with a manageable number of variables. This will reveal a range of possible outcomes at a very local level and, at the very least, allow for some hypotheses concerning major factors influencing diversity/function relationships. Are these still relevant when we multiply local patches and allow them to interact? A definitive answer will have to come from field studies on stream reaches and streams, conducted over several seasons. But potential patterns and how they emerge might best be studied by an approach variously labeled cellular automata, or individual-based modeling, or dispersed intelligence (Caswell, 1978; Huston, 1979; Halley et al., 1994, 1996; Wilensky and Resnick, 1999). Individuals (spores, colonies, leaves) can be represented as independent agents and provided with simple sets of rules. By increasing their numbers and defining certain parameters of their environment and how they respond to them, we may at least be able to define boundary conditions for likely outcomes. The same approach has proven that simple rules applied to large numbers of individual agents result in amazingly complex and realistic societal phenomena (Epstein and Axtell, 1996; Axelrod, 1997).

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4

Marine Fungal Communities

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4.1 THE CONCEPT OF MARINE FUNGI

Nearly three quarters of the Earth's surface area is covered by oceans. Seawater contains approximately 3.5% total dissolved salts (t.d.s.), mainly NaCl. Not all the fungi isolated from these environments are considered marine fungi in the classical definition. The definition of marine fungi is problematic by nature, as it is bordered by ecological and physiological requirements and not by taxonomic relation. The contemporary definition coined by Kohlmeyer and Kohlmeyer (1979) divides marine fungi into obligate species, which exclusively grow and sporulate in a marine (or estuarine) habitat, and facultative species, which are able to grow and possibly also sporulate in the marine environment but are found in terrestrial and freshwater milieus. A suggestion was made that the ability to germinate and to form mycelium under natural marine conditions should be used as a criterion for the definition of a marine fungus (Hyde et al., 2000).

Marine fungi are distinct in their physiology, morphology, and adaptation to an aquatic habitat (Meyers, 1996). To date, publications describe 467 higher marine fungi from 244 genera. They may be divided into a majority of Ascomycota (97%), a few Basidiomycota (~2%), and anamorphic fungi (< 1%). The largest order of marine ascomycetes is the Halosphaeriales, with some 45 genera and 119 species (Table 4.1).

4.2 THE ORIGIN OF MARINE FUNGI

The dominant method of identifying species is by morphological characters (Hawksworth et al., 1995). As widespread as this diagnosing method is, it may not be suitable for diagnosing evolutionarily meaningful species in fungi (Taylor et al., 2000). Another prob-

Table 4.1 Higher Fungi Recovered from the Marine Environment

Division	Order	Family	Genus [No. of Species]
Ascomycota	Arthoniales	Roccellaceae	*Halographis* [1]
	Chaetothyriales	Herpotrichiellaceae	*Capronia* [1]
	Diaporthales	Melanconidaceae	*Hypophloeda* [1]
		Valsaceae	*Cytospora* [1], *Diaporthe* [1], *Gloeosporidina* [1], *Gnomonia* [1], *Phomopsis* [1]
		Incertae sedis	*Pedumispora* [1]
	Dothideales	Botryosphaeriaceae	*Amarenographium* [1], *Amarenomyces* [1], *Diplodia* [1]
		Dothideaceae	*Scirrhia* [1]
		Planistromellaceae	*Loratospora* [1]
		Incertae sedis	*Belizeana* [1], *Biatriospora* [1], *Capillataspora* [1], *Heleiosa* [1], *Helicascus* [2], *Lineolata* [1], *Monodictys* [1], *Paraliomyces* [2], *Passeriniella* [2], *Salsuginea* [1], *Thalassoascus* [3]
	Halosphaeriales	Halosphaeriaceae	*Aniptodera* [8], *Anisostagna* [1], *Antennospora* [2], *Appendichordella* [1], *Arenariomyces* [1], *Bathyascus* [4], *Bovicornua* [1], *Carbosphaerella* [2], *Ceriosporopsis* [5], *Chadefaudia* [6], *Corallicola* [1], *Corollospora* [17], *Cucullosporella* [1], *Etheirophora* [3], *Haligena* [2], *Halosarpheia* [17], *Halosphaeria* [3], *Halosphaeriopsis* [1], *Iwilsoneilla* [1], *Kohlkmeyeriella* [1], *Lanspora* [1], *Lautisporopsis* [1], *Lignincola* [4], *Limacospora* [1], *Luttrellia* [1], *Marinospora* [2], *Moana* [1], *Nais* [2], *Naufragella* [2], *Nautosphaeria* [1], *Nereiospora* [2], *Nimbospora* [3], *Nohea* [1], *Ocostaspora* [1], *Ondiniella* [1], *Ophiodeira* [1], *Remispora* [6], *Sigmoidea* [2], *Thalassogena* [1], *Tirispora* [1], *Trailia* [1], *Trichomaris* [1], *Tunicatispora* [1], *Varicosporina* [2]
	Helotiales	Dermateaceae	*Laetinaevia* [1]
		Vibrisseaceae	*Vibrissea* [1]
	Hyaloscyphaceae	Helotiales	*Lachnum* [1]
	Hypocreales	Bionectriaceae	*Heleococcum* [1], *Hydropisphaera* [1], *Kallichroma* [2], *Pronectria* [1]
		Nectriaceae	*Tubercularia* [1]

		Incertae sedis	*Halonectria* [1], *Payosphaeria* [1]
	Hysteriales	Hysteriaceae	*Gloniella* [1]
	Laboulbeniales	Laboulbeniaceae	*Laboulbenia* [1]
	Lecanorales	Dactylosporaceae	*Dactylospora* [3]
	Lulworthiales	Lulworthiaceae	*Lindra,* [6], *Lulworthia* [10]
	Mycosphaerellales	Mycosphaerellaceae	*Ascochyta* [2], *Cladosporium* [1], *Clavariopsis* [1], *Mycosphaerella* [4], *Pharcidia* [3], *Sphaerulina* [1]
Ascomycota	Onygenales	Gymnoascaceae	*Gymnascella* [1]
	Patellariales	Patellariaceae	*Banhegyia* [1]
	Phyllachorales	Phyllachoraceae	*Polystigma* [1]
		Incertae sedis	*Didymella* [5], *Haloguignardia* [5], *Mangrovispora* [1], *Phycomelaina* [1]
	Pleosporales	Arthopyreniaceae	*Arthopyrenia* [1]
		Cucurbitariaceae	*Camarosporium* [2]
		Didymosphaeriaceae	*Didymosphaeria* [1], *Verruculina* [1]
		Leptosphaeriaceae	*Coniothyrium* [1], *Leptosphaeria* [6], *Phoma* [3]
		Lophiostomataceae	*Ascocratera* [1], *Lophiostoma* [2], *Massariosphaeria* [1], *Quintaria* [1], *Keissleriella* [1], *Massarina* [9]
		Melanommataceae	*Acrocordiopsis* [2], *Astrosphaeriella* [2], *Bicrouania* [1], *Caryosporella* [1], *Platystomum* [1], *Trematosphaeria* [2]
		Phaeosphaeriaceae	*Carinispora* [2], *Lautitia* [1], *Microsphaeriopsis* [1], *Paraphaeosphaeria* [1], *Phaeosphaeria* [11]
		Pleosporaceae	*Alternaria* [1], *Drechslera* [1], *Exserohilum* [1], *Falciformispora* [1], *Kirschsteiniothelia* [1], *Tremateia* [1], *Wettsteinia* [1], *Decorospora* [1], *Pleospora* [5], *Stemphylium* [2]
		Incertae sedis	*Stagonospora* [3]
	Pyrenulales	Massariaceae	*Aigialus* [4]
		Requienellaceae	*Pyrenographa* [1]
		Xanthopyreniaceae	*Pyrenocollema* [1]
	Rhytismatales	Incertae sedis	*Tiarosporella* [1]
	Sordariales	Chaetomiaceae	*Papulaspora* [1]
		Chaetosphaeriaceae	*Chaetosphaeria* [1]

Table 4.1 Higher Fungi Recovered from the Marine Environment (Continued)

Division	Order	Family	Genus [No. of Species]
		Lasiosphaeriaceae	*Biconiosporella* [1], *Zopfiella* [2]
		Nitschkiaceae	*Groenhiella* [1]
		Incertae sedis	*Nipicola* [2], *Savoryella* [5]
	Spathulosporales	Hispidicarpomycetaceae	*Hispidicarpomyces* [1]
		Spathulosporaceae	*Retrostium* [1], *Spathulospora* [5]
	Verrucariales	Verrucariaceae	*Mycophycias* [2]
			Verrucaria [1]
	Xylariales	Cainiaceae	*Atrotorquata* [1]
		Clypeosphaeriaceae	*Apioclypea* [1], *Ommatomyces* [1]
		Diatrypaceae	*Cryptosphaeria* [1], *Eutypella* [1], *Eutypa* [1]
		Hyponectriaceae	*Frondicola* [1], *Phragmitensis* [1], *Physalospora* [1]
		Xylariaceae	*Anthostomella* [9], *Halorosellinia* [1], *Hypoxylon* [2]
		Incertae sedis	*Adomia* [1], *Arecophila* [1], *Fasciatispora* [1], *Lanceispora* [1], *Linocarpon* [6], *Neolinocarpon* [1], *Oxydothis* [2], *Phomatospora* [6]
	Incertae sedis	Hypsostromataceae	*Manglicola* [1]
		Koralionastetaceae	*Koralionastes* [5]
Ascomycota	Incertae sedis	Lautosporaceae	*Lautospora* [2]
		Leotiomycetidae	*Amylocarpus* [1]
		Magnaporthaceae	*Buergenerula* [1]
			Gaeumannomyces [1]
			Juncigena [1]
		Mastodiaceae	*Mastodia* [1], *Turgidosculum* [1]
		Melaspileaceae	*Melaspilea* [1]
		Orbiliaceae	*Dwayaangam* [1], *Orbilia* [1]
		Papulosaceae	*Papulosa* [1]

		Thelenellaceae	*Julella* [1]
		Zopfiaceae	*Caryospora* [2], *Halotthia* [1], *Pontoporeia* [1]
		Incertae sedis	*Abyssomyces* [1], *Anguillospora* [1], *Aquamarina* [1], *Aropsiculus* [1], *Asteromyces* [1], *Biflua* [1], *Blodgettia* [1], *Chaetomastia* [1], *Cirrenalia* [7], *Clavatospora* [1], *Crinigera* [1], *Cryptovalsa* [2], *Cumulospora* [1], *Cytoplacosphaeria* [1], *Dictyosporium* [1], *Dinemasporium* [1], *Dryosphaera* [2], *Eiona* [1], *Epicoccum* [1], *Glomerobolus* [1], *Hapsidascus* [1], *Helicorhoidion* [1], *Hymenopsis* [1], *Hyphopolynema* [1], *Koorchaloma* [1], *Marinosphaera* [1], *Marisolaris* [1], *Nypaella* [1], *Oceanitis* [1], *Orbimyces* [1], *Orcadia* [1], *Periconia* [2], *Phialophorophoma* [1], *Plectophomella* [1], *Pleurophomopsis* [1], *Pontogeneia* [7], *Rhabdospora* [1], *Rhizophila* [1], *Robillarda* [1], *Saccardoella* [3], *Scolecobasidium* [2], *Sporidesmium* [1], *Sulcospora* [1], *Swampomyces* [4], *Tirisporella* [1], *Torpedospora* [2], *Trichocladium* [6], *Xylomyces* [1], *Zalerion* [2]
Basidiomycota	Agaricales	Marasmiaceae	*Calathella* [1], *Physalacria* [1]
		Niaceae	*Nia* [3]
	Cantharellales	Botryobasidiaceae	*Allescheriella* (anamorph) [1]
	Polyporales	Atheliaceae	*Digitatispora* [2]
		Cyphellaceae	*Halocyphina* [1]
	Urocystales	Melanotaeniaceae	*Melanotaenium* [1]
	Incertae sedis		*Mycaureola* [1]
Anamorphic fungi			*Botryophialophora* [1], *Floricola* [1], *Macrophoma* [1]

lem is encountered when contrasting ecological differentiation and the evolutionary development of marine fungi.

The largest order of marine fungi, the Halosphaeriales, is polyphyletic. They show independent terrestrial origins (Spatafora et al., 1998). Additional study of this order was performed using partial 18S rDNA with four *Halosarpheia* spp., *Lignincola laevis*, and *Nais inornata.* The species examined did not form a monophyletic group. The marine *Halosphaeriales* formed a subclade with taxa from the *Microascales*. Data indicate that *L. laevis* and *N. inornata* may be congeneric (Kong et al., 2000).

Even at the genera level taxonomic relations are uncertain. The genus *Halosarpheia* is populated by species with the morphological trait of unfurling ascospore appendages (Baker et al., 2001). This character is likely to be of an adaptive nature. Phylogenetic analysis of 18S rDNA sequences demonstrate polyphyly of the genus, with *H. fibrosa*, the type of the genus, resolved separately from all other species of *Halosarpheia* analyzed. Though morphologically similar, *H. retorquens*, *H. viscosa*, *H. heteroguttulata*, and *H. lotica* were discovered to be distinct species. Some morphological characters used to delineate species within *Halosarpheia*, such as ascospore guttulation pattern and number of ascospore appendages, may actually reflect generic differences that have been overshadowed by convergent evolution in appendage type (Anderson et al., 2001). Consequently, it is probable to infer that marine fungi have diverse terrestrial origins. Adaptations to the maritime environment and convergent evolutionary processes have led to the ecological affiliation that is seen today.

4.3 THE MARINE COMMUNITY

The characterization of a community or, at the broader sense, an ecosystem has three attributes: composition, structure, and function. Methodical inventories in the form of data collections are useful, most importantly because they serve as the only direct evidence of species distributions. However, collecting bias has been demonstrated for most areas of the world, as data are often geographically, temporally, or taxonomically incomplete (Funk and Richardson, 2002).

Local biological communities are made up of species, each of which has its own particular relationship with the environment. The relationship between biodiversity and individual ecosystem processes is often asymptotic, saturating at relatively low levels, with some species contributing more strongly than others (Schwartz et al., 2000).

The most straightforward approach to determining the links between species diversity and ecosystem function is to isolate organisms considered to be important in ecosystem function and to assess their potential ecological impact through laboratory-based, physiological studies.

The term *marine environment* encompasses a diverse list of niches. From the brackish water of estuaries to the deep-sea areas, they differ in both abiotic and biotic characteristics. The fungal diversity of a marine environment is affected by temperature, salinity, substrata, and propagules availability (Jones, 2000). At the Seventh International Marine and Freshwater Mycology Symposium held in Hong Kong in 1999, it was proposed to use terms such as *maritime*, *halotolerant*, *estuarine*, *resident*, and *native*, in addition to *marine fungi*. Today the majority of fungi classified as obligate marine are parasites on plants and animals, symbionts in lichenoid association with algae, and saprobes on dead organic matter of plant or animal origin. In their life cycle, these fungi are associated with organisms that constantly live in oceans and estuaries. The following sections will describe species composition, structure of different habitats, and factors influencing the whole of the marine fungal community.

4.4 BIOGEOGRAPHY

Most marine fungi show one of four patterns of distribution: arctic, temperate, tropical, or cosmopolitan. While the majority of fungi may be grouped to pantemperate and pantropical, there is little evidence of species being restricted to countries or continents (Jones, 1993). Of the approximately 500 species of marine fungi that have been described, at least 135 are found in the tropics (Jones, 1993). While there is no evidence to suggest that there are more tropical than temperate marine fungi, the contrary is also true. Although relatively few marine fungi have been named, the group should not be excluded from global estimates of fungal diversity.

Water temperature is the most important factor in controlling the geographical distribution of marine fungi (Jones, 1993). Tropical currents or colder currents often cross boundaries and influence the mycobiota therein. Where intermediate sea temperatures occur there is often a mixture of temperate and tropical fungi (Jones, 1993). This would be expected as these fungi are dispersed by separate propagules or on wood growing in the sea, and therefore sea masses present no barriers to their dispersal.

Geographical regions can be divided by species that are typically tropical (e.g., *Antennospora quadricornuta* and *Halosarpheia ratnagiriensis*), temperate (e.g., *Ceriosporopsis trullifera* and *Ondiniella torquata*), and arctic (e.g., *Spathulospora antartica* and *Thraustochytrium antarticum*). The intertidal mangrove species *Halosarpheia fibrosa* and *Halosarpheia marina* may have a subtropical distribution (Hyde and Lee, 1995). Some marine fungi are cosmopolitan (Jones, 1993), like *Ceriosporopsis halima*, *Lignincola laevis*, *Corollospora maritime*, and *Halosphaeria appendiculata*, which are more or less common in temperate and tropical seas (Jones, 2000). In most cases, the occurrence of a fungus in a particular habitat is related to water temperatures and substrata. The effect of the latter is particularly striking, as the fungi occurring on submerged wood in the open sea usually differ from those on intertidal mangrove wood. In turn, these fungi differ from those occurring on leaves or algae (Hyde et al., 1989a).

4.5 BIODIVERSITY

Marine mycelial decomposers (eumycotes — true fungi, oomycotes — pseudofungi) are highly adapted for capture of solid substrates by pervasion and digestion from within. Over 90% of the higher marine fungi use woody and herbaceous substrata (Figure 4.1). They are major decomposers of the above-mentioned substrata that enter marine ecosystems. Thus, they exert their influence in areas of large input of litter of vascular plants, especially at some types of terrestrial/marine ecosystemic interfaces (ecotones) (Hyde et al., 1989a). Unavailability of methods easily used by general microbial ecologists has hampered progress in the study of marine mycelial decomposers, leaving numerous pockets of difficulty in this regard (Newell, 1994). Refined methods for measuring fungal mass and productivity have begun to enable the realization of the impacts of fungi in marine ecotones (Gessner and Chauvet, 1993; Gessner and Newell, 1997; Fell and Newell, 1998). The generally held belief in the standing-decay system of saltmarsh grasses has been refuted: it was shown that fungal mass can account for virtually all of the nitrogen present at some points in the standing-decay period (Newell and Porter, 2000). Another opinion about marine fungi has also been reversed: ascomycetes of a saltmarsh grass (smooth cordgrass) clearly do digest lignocellulose under natural-decay circumstances (Gessner, 1980).

Much more work is needed to clarify the situation, but at present, it appears that major types of marine ecotones (e.g., saltmarshes and mangroves) differ sharply in the

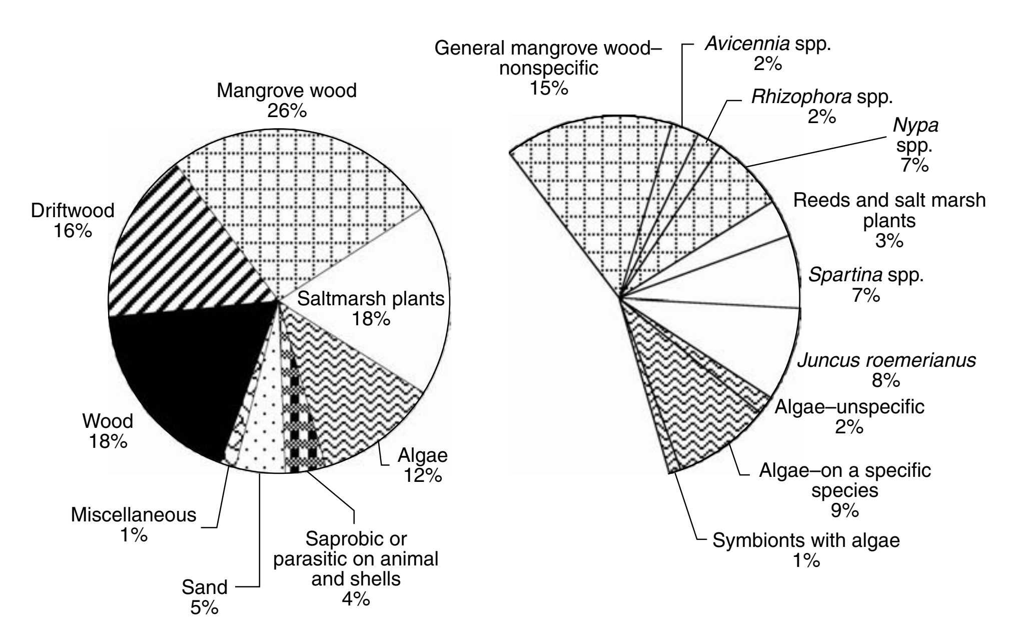


Figure 4.1 Different substrates used by higher marine fungi. Given are the percentages of the total numbers of higher marine fungi. Left chart: General type of material. Right chart: Major specific hosts of mangroves, algae, and saltmarsh plants. Data compiled from references mentioned within.

balance among major groups of decomposers (eumycotes, oomycotes, and bacteria) with regard to their utilization of vascular plant litter (Newell, 1996). In saltmarshes, microbial production in standing-grass litter is strongly dominated by fungi (Newell, 2001a, 2001b), while oomycotes do not show evidence of a substantial role in decomposition. By contrast, in mangroves, submerged fallen leaves appear to support minor fungal occupancy (Newell and Fell, 1992), yet ubiquitous and rapid occupancy by oomycotes (especially *Halophytophthora vesicula*) (Newell, 1992).

Although total biodiversity is not affected by the available habitats, species composition is. For example, members of the Halosphaeriales commonly occur on submerged timber, while intertidal mangrove wood supports a wide range of Loculoascomycetes (Jones and Alias, 1997). Some marine fungi also sporulate on sand grains and on hard material such as coral (Jones and Mitchell, 1996). The availability of substrata for colonization greatly affects species richness. Mature mangroves yield a rich species diversity (Sarma and Hyde, 2001), while exposed shores or depauperate habitats support few fungi. For instance, five niches were examined in the coastal areas of Brunei: a rocky headland, a sandy beach, a man-made brackish lake, a healthy mangrove, and an oil-polluted mangrove. Although some fungi occurred throughout, species composition differed from one habitat to the next. The common marine fungi species at each habitat also varied. *Antennospora quadricornuta* was most common at the rocky headland, *Corollospora pulchella* at the sandy beach, and *Halosarpheia marina* at the brackish lake. In the mangroves, the most common species were *Halocyphina villosa* (healthy) and *Cirrenalia pygmea* and *Lulworthia grandispora* (oil polluted) (Hyde, 1989b).

The nature of a substratum can have a major effect on the fungi colonizing it, even from one timber species to the next (Hyde, 1990a). Lignocellulosic materials yield the greatest diversity, in contrast to a few species colonizing calcareous materials or sand

grains (Figure 4.1). Competition between fungi can markedly affect fungal diversity and species composition (Tan et al., 1995). Salinity is also important in affecting species composition. Many fungi occur primarily in fully saline waters (e.g., *Lindra inflata* and *Ondiniella torquata*); others are more frequent in brackish water (e.g., *Amylocarpus encephaloides* and *Aniptodera chesapeakenis*), while terrestrial and freshwater species may be able to grow at lower salinities (e.g., *Chytridium citriforme*, *Saprolegnia ferax*, and *Stachybotrys atra*) (Jones, 2000). In mangroves many species (e.g., *Halophytophthora* species) can tolerate great variation in salinity of the water (Leano et al., 2000).

A plethora of factors may govern the occurrence of marine fungi in a particular habitat or on a substratum. Some marine fungi are common in occurrence (e.g., *Ceriosporopsis halima*, *Lulworthia* spp., and *Zalerion maritimum* in temperate waters and *Antennospora salina*, *Antennospora quadricornuata*, and *Lulworthia grandispora* in the tropics), while others are rarely collected (e.g., *Orbimyces spectabilis* and *Torpedospora ambispinosa*). For the latter group, this may be due to seasonality (e.g., *Mycaureola dilsea* on *Dilsea edulis*) or temperature requirements (e.g., *Digitatispora marina* occurring during the winter months when water temperatures are below 10°C). For other species, there is no apparent reason to explain their infrequent occurrence. Many are subject to a consortium of factors operating together in controlling the biodiversity of fungi in the sea.

4.6 COASTAL HABITATS

Approximately 7% of the global land surface is covered with saline habitats (Ruiz-Lozano et al., 1996), which have remarkably similar plant communities and zonal distributions of species, depending on the salt concentration (Walter, 1968; Chapman, 2001). Saltmarshes, sea grass beds, and mangrove forests produce large amounts of dead plant material (litter), much of which enters the system as relatively large detrital particles. Allochthonous detritus, entering from rivers and the coastal ocean, typically occurs in smaller fractions and later stages of conditioning. In all marine ecotones, the organic matter is rapidly incorporated into a complex decomposer food web in which fungi are secondary producers. *Antennospora quadricornuata*, *Arenariomyces* species, *Corollospora* species, and *Torpedospora radiadata* are typical fungi of coastal waters (Jones, 2000). *Antennospora salina* is a dominant species from intertidal wood in open beaches (Patil and Borse, 2001). The genera *Arenariomyces*, *Corollospora*, and *Nereiospora* are often associated with grains of sand (Nakagiri and Tokura, 1987; Kohlmeyer and Volkmann-Kohlmeyer, 1987, 1989). Other associations may be uncovered from seaweed (Iberian coasts): *Asteromyces cruciatus*, *Corollospora intermedia*, *Dendryphiella arenaria*, *Dendryphiella salina*, and *Varicosporina ramulosa* (Genilloud et al., 1994). *Antennospora salina* may be identified from intertidal wood in open beaches (Patil and Borse, 2001). The diversity of marine fungi on intertidal wood collected from beaches, islands, and harbor locations in the west coast of India revealed five frequent fungi: *Antennospora quadricornuta*, *Clavatospora bulbosa*, *Crinigera maritima*, *Periconia prolifica*, and *Torpedospora radiata* (Prasannarai and Sridhar, 2001).

4.6.1 Saltmarsh Habitats

Bays and estuaries, and their surrounding saltmarshes and mudflats, are among the most productive systems of the biosphere. In 1968, Odum (Teal, 1962; Odum and de la Cruz, 1967) developed the outwelling paradigm that stated that saltmarshes export organic matter, thereby enhancing the whole food web of the adjacent coastal waters. Saltmarshes constitute dynamic environments that present characteristics of both marine and terrestrial

systems. Often estuaries have water ranging from nearly fresh to same as oceanic: ~3.5% t.d.s. According to literature data, only a few animal species have the ability to feed directly on the living plant material, so fungi and bacteria seem to be the principal competitors for the organic substrates. Microbial production in standing-grass litter is dominated by fungi, mainly by ascomycetes.

One of the most explored saltmarshes is along the northeast coast of North America. The ecology of the mycobiota is relatively well understood. In this location, the saltmarsh plants occupy the major part of the intertidal area and are immersed at each tide (McKee and Patrick, 1988). In the southeastern coast saltmarshes, the smooth cordgrass *Spartina alterniflora* is a predominant vascular plant long believed to contribute considerably to energy flow in tidal marsh estuaries (Peterson and Howarth, 1987). The importance of fungi in the biogeochemical cycling of carbon and nutrients in coastal saltmarshes has been well established (Newell, 2001a). A number of ascomycetes are involved in the decomposition of standing-dead leaves of *S. alterniflora*, where productivity can reach 500 g/m^2 per annum and their living biomass can contribute as much as 20% to the dry biomass of decaying leaves (Newell, 1993). The potential levels of fungal production per unit of naturally decaying grass are similar in northern and southern marshes as tested from sites in Florida to Maine (Newell et al., 2000). Studies of the east coast of North America saltmarshes (many by Kohlmeyer and Volkmann-Kohlmeyer*) revealed numerous novel species. To date, there are 39 marine species on *Spartina* spp. and 50 described species on *Juncus roemerianus*, an important component of saltmarshes of the U.S. east coast, the Gulf of Mexico, and the Atlantic coast of southern America (Kohlmeyer and Volkmann-Kohlmeyer, 2002). *Trichocladium medullare* is one of the frequent species on saltmarsh *J. roemerianus* in North Carolina (Kohlmeyer and Volkmann-Kohlmeyer, 1995).

Saltmarsh fungi colonize senescing blades of the dominant primary producer, *S. alterniflora*, while the blades are still attached to the stem. As they carry out decomposition, fungi efficiently convert plant organic matter into fungal biomass (155 to 217 μg of fungal organic mass per gram of system organic mass) in the form of mycelia and reproductive structures (Newell and Porter, 2000; Newell, 2001a, 2001b). Further links from fungi to bacteria (Newell and Porter, 2000; Newell, 2001a) and animal shredders, as marsh macroinvertebrates that remove 20 to 50% of net fungal biomass (Graça et al., 2000), establish an important detritus-based food web in one of the most productive marine ecosystems.

The *S. alterniflora* decay system in southeastern U.S. saltmarshes is one of the few natural ecosystems for which fungal community composition is relatively well known. Using direct microscopic observation, several species of ascomycetes have been identified as major decomposers of *S. alterniflora* blades (Newell, 2001a, 2001b). The two most regularly recorded species (78% of all ascospores expelled from decaying blades over a 3-year period and occurrence of ascomata in 66 to 77% of blades examined) are *Phaeosphaeria spartinicola* and *Mycosphaerella* sp. 2 (Kohlmeyer and Kohlmeyer, 1979). Both species are involved in lysis of lignocellulosic components of the blades (Bergbauer and Newell, 1992; Newell et al., 1996; Newell and Porter, 2000). Other ascomycetes, such as *Phaeosphaeria halima* and *Buergenerula spartinae*, occur in about 2 to 40% of blades examined (Newell, 2001b). In the leaf sheaths and true stems of decaying *S. alterniflora,* distinctly different ascomycetes from those of blades are detectable (e.g., *Lachnum spartinae* in sheaths) (Newell and Porter, 2000).

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* Kohlmeyer and Volkmann-Kohlmeyer, 1993–2002, especially the "Fungi on *Juncus roemerianus*" series, 1–16.

Additional confirmation of the hierarchal structure was made by characterization of the ascomycete community colonizing decaying *S. alterniflora* blades by analysis of internal transcribed spacer (ITS) regions of fungal rRNA genes. Three species of ascomycetes — *Mycosphaerella* sp. 2, *Phaeosphaeria spartinicola*, and *Phaeosphaeria halima* — dominated the *S. alterniflora* blades at two stages of decay (early and late) (Buchan et al., 2002).

In contrast to the east coast of North America, in most European bays and estuaries, saltmarsh plants are confined to the uppermost part of the intertidal zone where they are immersed only periodically by spring high tides (Beeftink, 1977). Indeed, plants that occur in European saltmarshes such as *S. alterniflora*, the dominant species of North American marshes, are able to tolerate being covered occasionally by saline estuarine water, but not twice a day.

The macrotidal Mont Saint-Michel Bay (gulf of Normandy, France) has been the location for studies on organic matter and nutrient fluxes between saltmarshes and marine waters. Most of the organic matter was trapped *in situ*, processed by fungi and bacteria, and then released seaward via tidal fluxes, groundwater, and runoff as particulate and nutrients. The flats are only occasionally submerged. Fungal activity was centered in the high marsh, where the flats are covered by plants (Lefeuvre et al., 2000). Examination of seasonal changes in the microbial community of an intertidal saltmarsh in northwest England showed a brief late-summer peak in the fungal biomass (Keith-Roach et al., 2002).

Spartina maritima is a dominant plant species in the Mondego saltmarsh on the western coast of Portugal, and it plays a significant role in estuarine productivity. Fungal biomass increased in wet months, with a maximum in January in standing-decay leaves and in naturally detached leaves in December. Biomass decreased greatly in summer. The biomass trends were reversed to mean salinity values. Comparative ergosterol concentrations associated with standing-decay and naturally detached leaves suggest that fungal activity was more important before leaf fall (Castro and Freitas, 2000).

In saltmarshes of Kuwait and Egypt, the most common species were all terrestrial species, such as *Aspergillus niger*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Alternaria alternata* (Moustafa, 1975; Abdel-Hafez et al., 1978). Marine *Ascochyta salicorniae* and *Kallichroma tethys* were dominant in the saltmarsh habitats of the Gujarat coast (Borse et al., 2000).

Hence, while the North American saltmarsh is well researched, the field is still open in many localities of Europe and the temperate zones, especially as to the part taken by fungi decomposition.

4.6.2 Mangrove Habitats

Mangroves, the climax formation of hydrohalophytes belonging to several plant families, inhabit tropical and subtropical estuarine or marine saltmarshes. Mangrove forests are considered open interface ecosystems connecting upland terrestrial and coastal estuarine ecosystems (Lugo and Snedaker, 1974). Contributors to the geoaquatic food chain, mangrove forests are important for biomass production and coastline protection.

Mangrove fungi may be found in the subtropics and warm temperate regions, but its species diversity is higher in the tropics (Hyde and Lee, 1995). The greater number of fungi may be representative of the higher mangrove tree diversity (Jones and Alias, 1997). There seems to be no discernible difference between mangrove fungi reported in the subtropics and those found in tropical areas. This is also true for frequently recorded fungi (Sarma and Hyde, 2001). Marine mycobiota reported from warm temperate mangroves of Australia is only a little different from the mycobiota reported from the tropics (Hyde, 1989c, 1990b). Fungi inhabiting mangrove habitats differ in their distribution. It seems

that mangrove fungi diversity is dependent on the diversity, age, and abiotic factors, such as salinity and tidal range, of the mangrove stand (Hyde and Lee, 1995). The species *Hypoxylan oceanicum*, *Kallichroma tethys*, and *Leptosphaeria astraliensis* are generally found on mangrove substrata (Jones and Hyde, 1990).

As with many other organisms, only a few fungi predominate on a given substratum or a given habitat. Although a core group of fungi can be recognized from mangroves at different geographic locations, the very frequent fungi seem to differ from one site to another. The very frequent fungi overlap between the Pacific and Indian Oceans. However, little data are available on the frequency of occurrence of mangrove fungi from the Atlantic Ocean. More information on frequently occurring fungi in mangroves may be found in the review by Sarma and Hyde (2001).

The mycobiota colonizing the ectorhizosphere–rhizoplane zone of saltmarsh mangrove species *Avicennia marina*, *Halocnemum strobilecium*, *Zygophyllum album*, *Zygophyllum coccineum*, *Zygophyllum simplex*, *Arthrocnemum macrostachum*, and *Limonastrum monopetalum* were investigated along the Red Sea coast of Egypt. The rhizosphere contained both xerotolerant and nontolerant terrestrial species. *Scolecobasidium arenarium* was the only recorded marine species (El-Morsy, 1999). This habitat has a higher salinity than oceans and diminished shrub-like trees, a factor affecting the mycobiota.

A survey of marine fungi present on dead prop roots of *Rhizophora mucronata* was conducted at the Kosi system, Durban Bayhead, and Mtata River, South Africa. The survey revealed 38 species of fungi. *Kallichroma* spp. and *Phoma* spp. were present in high numbers at all three estuaries; *Leptosphaeria australiensis* and *Swampomyces triseptatus* occurred frequently at Kosi and Mtata; and *Dactylospora haliotrepha* was common at Bayhead and Mtata. Species diversity was lower than recorded for most comprehensive surveys in tropical Southeast Asia. However, the colonization percentage and frequencies of occurrence of fungi on wood indicate that marine fungi have an important ecological role to play in local mangrove communities. More information is required not only from South Africa but also from the tropical African east coast (Steinke, 2000).

An object of interest for mangrove fungi is host and substratum specificity. Some fungi show specificity to a particular host. Furthermore, some fungi occur more commonly on one or two hosts (Sarma and Hyde, 2001). In a study conducted on five mangrove tree species, *Avicennia alba*, *Rhizophora apiculata*, *Rhizophora mucronata, Sonneratia alba*, and *Xylocarpus granatum*, in the intertidal zone in Brunei, it was found that each tree species had a different fungal community. Some fungi were specific and limited to single tree species, e.g., *Xylocarpus*, *Caryospora mangrovei*, *Avicennia*, *Aigialus mangrovis*, and *Eutypa* sp. Others were nonspecific and developed on several tree species, e.g., *Hypoxylon oceanicum*, *Leptosphaeria australiensis*, and *Savoryella lignicola* (Hyde, 1990a). In another study, the examination of decaying mangrove materials collected from Godavari and Krishna deltas (Andhra Pradesh), east coast of India, resulted in the identification of 88 fungi. The maximum number of species (64) was recorded from *Rhizophora apiculata*, followed by *Avicennia officinalis* (55) and *Avicennia marina* (45). *Verruculina enalia* was recorded on all the host plants examined. *Halorosellinia oceanica*, *Hypoxylon* sp., *Lophiostoma mangrovei*, *Lulworthia* sp., *Lulworthia grandispora*, and *Hysterium* sp. *Trichocladium achrasporum* were isolated from more than half of the examined host plants (Sarma and Vittal, 2001). In another sampling in the same location, of 85 species, 22 were recorded only from *Avicennia* and 20 only from *Rhizophora*. *Verruculina enalia* was the only very frequent fungus encountered on both hosts (Sarma et al., 2001a).

The fungal diversity on prop roots, seedlings, and wood of *Rhizophora apiculata* and on wood, roots, and pneumatophores of *Avicennia* spp. has been investigated. Decomposing substrata were collected from the deltaic mangroves of Godavari and Krishna

Rivers, in the east coast of India. The number of fungi recorded on prop roots of *Rhizophora apiculata* (61) was much greater than those on wood (24) and seedlings (21). Thirty-two species were recorded exclusively on prop roots. The number of fungal species recorded on *Avicennia* wood (61) was much greater than those on pneumatophores (14) and roots (17). Forty-two fungi were recorded only on wood. Differences in frequency of occurrence on assorted substrates of a host appeared, with many of the fungi common to all three substrata of the respective hosts, including *Dactylospora haliotrepha*, *Leptosphaeria australiensis*, *Lophiostoma mangrovei*, *Lulworthia* sp. *Phomopsis mangrovei*, and *Saccardoella rhizophorae. Verruculina enalia* was frequent on all three substrata in both mangrove species (Sarma and Vittal, 2000).

Nypa palm is a common mangrove species in Southeast Asia where it can form extensive stands (Tomlinson, 1986). Study of the mycobiota of intertidal *Nypa* palm reported over 40 intertidal fungal species (Hyde, 1991c, 1992b, 1992c; Hyde and Alias, 2000). Most of the fungi found on *Nypa palmare* are intertidal and do not appear to occur on nonpalm hosts; the evidence indicates that there are probably more than 40 species that are unique to *Nypa fruticans*. The largely distinct mycota found on *Nypa* may be accounted for by host specifity, or a second explanation may be the lower salinity preference of this host (Tomlinson, 1986). The question of host specificity in general mangrove fungi is still unresolved and requires further study.

Mangrove fungi tend to be restricted to a vertical region, probably as affected by tidal range and rate of immersion. Fungi were found to be vertically zoned on decayed samples of *Rhizophora apiculata* in Brunei: some were limited to the upper (high water mark) (e.g., *Hypoxylon oceanicum*, *Savoryella lignicola*) or lower (e.g., *Antennospora quadricornuta*, *Thalassogena sphaerica*) levels, while only two (*Cirrenalia pygmea* and *Lulworthia* sp.) were present throughout the tidal range. The greatest number of species was collected from above mean tide (Hyde, 1990c). Similar results were seen in studies on the vertical distribution of marine fungi in an *R. apiculata* mangrove stand in Morib, Selangor. The fungi were limited to the upper level (e.g., *Pyrenographa xylographoides*, *Julella avicenniae*, and *Aigialus grandis*) or lower level (e.g., *Trichocladium achrasporum* and *Trichocladium alopallonellum*), while only five species showed a broader distribution, being present at all levels (e.g., *Leptosphaeria australiensis*, *Halocyphina villosa*, *Cryptovalsa* sp., *Lulworthia grandispora*, and *Lulworthia* sp.). The greatest diversity of marine fungi was collected from the middle level. The upper and middle levels were the most similar in terms of species composition. Fungi with certain characteristics were also limited to particular levels; for example, carbonaceous and superficial ascomata were confined above mean tide, while membranous walls and immersed ascomata were common for fungi below mean tide level (Alias and Jones, 2000b). No information is available on this topic for most parts of the world or most tidal ranges.

Succession of fungi on wood is difficult to discern, but a clear pattern of colonization may be assessed. Fungal colonization was noted on test poles of *Rhizophora apiculata* and *Xylocarpus granatum* exposed in the intertidal region of Kampong Kapok mangrove, Brunei. *Halosarpheia minuta* was an early colonizer on *R. apiculata* and *Lulworthia* sp. of *X. granatum* (Hyde, 1991a). Fungal colonization of wood is affected by the presence or absence of bark (Leong et al., 1991). Patterns were determined by examination of test blocks of *Avicennia marina* and *Bruguiera parviflora* at Kuala Selangor mangrove stand. A similarity index showed the species composition on *A. marina* and *B. parviflora* to be relatively similar at each stage of colonization. Lower numbers of fungi and percentage of colonization were observed at the early stage than at the intermediate and late colonization stages. Early colonizers (6 to 18 weeks) on both timbers and at all stands included *Halosarpheia marina*, *Halosarpheia retorquens*, *Lignincola laevis*, *Lignincola longiros-*

tris, and *Lulworthia grandispora*. Intermediate colonizers (26 to 54 weeks) included *Dictyosporium pelagicum*, *Halocyphina villosa*, *Halosarpheia ratnagiriensis*, *Periconia prolifica*, *Savoryella lignicola*, *Trichocladium achrasporum*, *Trichocladium alopallonellum*, and *Verruculina enalia*. Late colonizers (60 to 96 weeks) were *Aigialus parvus*, *Leptosphaeria australiensis*, *Nais glitra*, *Quintaria lignatilis*, *Saccardoella marinospora*, and *Tirispora unicaudata* (Alias and Jones, 2000a).

Split wood blocks of *Bruguiera gymnorrhiza* and *Rhizophora mucronata* were submerged in the intertidal zone at five mangrove sites in Mauritius. No distinct colonization patterns were common to all study sites or either of the hosts. *Cumulospora marina* was strictly an early colonizer. *Cirrenalia pygmea* and *Lulworthia* species were more common as early colonizers. *Lignincola laevis* was more common as an early to intermediate colonizer. *Periconia prolifica* occurred throughout the experimental period at three of the sites, while *Dactylospora haliotrepha* and *Coelomycete* sp. were late colonizers. A relatively high percentage of the fungi colonizing the test blocks were anamorphic fungi (Poonyth et al., 2001). Subsequently, no definite stage may be registered to a fungal species; colonization patterns seem to be dependent on a combination of factors: substrate type, host species, and abiotic conditions.

It is clear that the knowledge of mangrove fungi ecology is partial at all levels (Hyde and Lee, 1995). Information is lacking from African mangroves, South America, most of the Pacific Ocean, and north and west Australia. Sarma and Hyde (2001) suggested a standardized protocol to enable the current gaps to be bridged. The studies to follow are yet to be published.

4.7 OCEANIC WATERS

The deep sea constitutes one of the least explored habitats on Earth, especially considering its volume as an environment. More than half of the Earth's surface is covered with water, ranging in depth from $3 \cdot 10^3$ to $6 \cdot 10^3$ m, with an average depth of approximately $3.2 \cdot 10^3$ m (Gage and Tyler, 1991). The deep-sea conditions include high hydrostatic pressures; low temperatures; low nutrient concentrations, with the notable exception of hydrothermal vents; and complete darkness.

Several substrates, such as wood, particulate organic matter (POM), and chitin (exoskeletons of marine crustacea), could participate in transportation and nutrition of marine fungi and were shown to be degraded by autochthonous fungi even in greater depths of the oceans (Kohlmeyer, 1969; Kohlmeyer and Kohlmeyer, 1979; Kohlmeyer and Volkmann-Kohlmeyer, 1988). Mycelial fungi have a negligible presence in the marine plankton (Newell, 1996). Association with resource-rich particles benefits planktonic species in oligotrophic, open-ocean regimes. Colonies of the cyanobacterium *Trichodesmium* spp. in the Sargasso Sea harbored associated fungi. Fungal filaments formed very dense bundles concentrated around a few trichomes in the colony and seemed to preclude the growth of dense bacterial populations (Sheridan et al., 2002). Bacteria and fungi may compete for nutrients from the polysaccharide matrix, algal exudates, or dead cells (O'Neil and Roman, 1992).

The recognized deep-sea fungi are *Allescheriella bathygena* on driftwood, *Oceanitis scuticella*, and *Periconia abyssa*, isolated by Kohlmeyer (1977). Two filamentous fungi, *Aspergillus ustus* and *Graphium* sp., were isolated from calcareous animal shells at depths of 860 m in the Arabian Sea and 965 m in the Bay of Bengal. The strains were able to pass through an entire life cycle under deep-sea conditions (100 bar, 10 and 30°C) (Raghukumar and Raghukumar, 1998).

Experimental investigation performed on marine and terrestrial isolates of filamentous fungi (and yeasts) showed that hydrostatic pressure is a major limiting factor for growth and metabolic activity in the deep-sea environment (Gonda et al., 2000).

Hydrothermal vents are deep-sea oases. Molecular microbial ecology studies have revealed remarkable diversity in extreme hydrothermal marine environments. Eukaryotic diversity was characterized by using sequence comparisons of PCR-amplified ssr RNAs in hydrothermal vent environments of Guaymas Basin in the Gulf of California. Among the recovered sequences were some closely related to those from eukaryotes that are more generally distributed in marine environments, including fungi such as *Aspergillus flavus* and *Aureobasidium pullulans* (Edgcomb et al., 2002).

4.8 PSEUDOFUNGI (CHROMISTA)

Members of the Chromista may be encountered in nearly all the locations where fungi can be found. They play an important role in some environments, e.g., mangroves. Therefore, a short summary on this topic has been included in this chapter. Three phyla belong in Chromista: Hyphochytriomycota, Labyrinthulomycota, and Oomycota (Kirk et al., 2001). Species from Hyphochytriomycota may be isolated in freshwater or soil and are not relevant to the current discussion.

4.8.1 Oomycota

The Oomycota, although behaviorally similar, are biologically distinct from the other main fungal groups that comprise the kingdom Fungi (Corliss, 1994; Cavalier-Smith, 1998). The principal genus of marine oomycotes is *Halophytophthora*. This genus was created to reclassify the group of marine species previously assigned to *Phytophthora.* Differentiation lay in the sporangial apical structure, the mode of zoospore emission, and other morphological and cultural characters (Ho and Jong, 1990). By Kirk et al. (2003) all the species changed by Ho and Jong (1990), including the type species *H. vesicula* (Anastasiou & Churchl.) H.H. Ho and S.C. Jong, were reassigned from *Halophytophthora* to *Phytophthora.* In this assay, they are still considered to be *Halophytophthora.*

Halophytophthora with *Pythium*, and *Phytophthora* are part of 10 genera of Pythiaceae, the only family in the order Pythiales (Dick, 1990). The class Oomycotes also contains saprotrophic, aquatic, zoosporangium-producing genera, such as *Saprolegnia* (mainly fish pathogens), *Achlya*, and *Dictyuchus* of the order Saprolegniales. Phylogenetic relationships among species of oomycetes were examined on the basis of the ITS sequences of genomic rDNA, and *Halophytophthora* was grouped with *Pythium*, *Peronospora*, and *Phytophthora*, distant from genera in the Saprolegniales (Cooke et al., 2000).

Halophytophthora species (16) are commonly isolated from fallen mangrove leaves from early to late stages of decay. Observations on the growth and reproduction of *Halophytophthora* species at the Shiira River, Japan, revealed that they are well adapted to the environmental conditions of their respective habitats. Nearly all species (except *H. vesicula*) showed a preference for a specific host or location. The levels and fluctuations of water salinity and temperature, and substrate types were found to be important factors influencing the local, seasonal, and geographical distribution of *Halophytophthora* species (Nakagiri, 2000). They have a wide tolerance to varying levels of pH (6 to 9), salinity (from freshwater to marine), and temperature. They also produce abundant zoospores, are chemotactically attracted to decaying mangrove leaves, and can readily attach to suitable substrata (Leano et al., 2000). Once the zoospores, cysts, and cystospores were attached to the substratum, they could not be readily dislodged, and successful germination and

colonization followed (Leano et al., 2000). Halophytophthoras complete their occupation of submerged leaves (from attachment of zoospore cysts to release of zoospores from sporangia) early in the decomposition of leaves, before substantial entry into the leaves of labyrinthulids (predators) (Newell and Fell, 1996).

Halophytophthora rapidly occupy fallen mangrove leaves (100% frequency of occurrence after 24 to 30 h). Four *Halophytophthora* species are commonly found: *H. kandeliae*, *H. masteri*, *H. spinosa* var. *spinosa*, and *H. vesicular*. In temperate saltmarsh waters, *H. kandeliae* took the place of *H. spinosa* as co-occupier of leaves with *H. vesicula*. Two rare species, *H. bahamensis* and *H. epistomium*, originally described from subtropical mangrove environs were found in temperate saltmarsh samples (Newell and Fell, 1995).

Competition experiments were performed using precolonized leaves of red mangrove *Rhizophora mangle* with higher marine fungi, species of *Halophytophthora*, and bacterial films. The ubiquitous coastal-marine oomycote *H. vesicula* was found to be an able competitor vs. other halophytophthoras and vs. true fungi, except the fungi *Dendryphiella salina*, common in decaying drift material in high-intertidal zones. *H. spinosa* and *H. bahamensis* were weak competitors with true fungi and with *H. vesicula*. When bacterial films were present on leaves prior to access by halophytophthoras, the occupation frequency of halophytophthoras was sharply depressed (by about 70 to 90% within 48-h bacterial films), implying that in some types or parts of mangrove systems, submerged-leaf decomposition may sustain low levels of participation by halophytophthoras (Newell and Fell, 1997).

Zoosporic fungi of the Pythiaceae, especially *H. vesicula*, were present at high frequency (90%) on autumn-shed leaves both north and south of a mangrove–saltmarsh boundary (Florida–Georgia). Submerged, decaying leaves of mangroves (three species), other trees (including conifers), shrubs, and vines bore *H. vesicula. H. bahamensis* was also found north of the mangrove belt but at low frequencies (10%). *H. kandeliae* was found only within the mangrove environment. Leaves of intertidal grasses (*Spartina* and *Juncus*) yielded low frequencies (20%) of *Halophytophthora* spp., but *Pythium grandisporangium* exhibited frequencies of up to 40 to 43% on leaves of trees and of *Spartina alterniflora* (Newell, 1992).

4.8.2 Labyrinthulomycetes

The Labyrinthulomycetes, comprising thraustochytrids and labyrinthulids, are marine osmoheterotrophic, straminipilan protists that have been isolated from a variety of habitats all over the world (Raghukumar, 2002). Thraustochytrids closely resemble zoosporic fungi in their morphology. Indeed, they were originally classified under the class Oomycetes of the division Mastigomycotina among Fungi, based on their biflagellate zoospores (Sparrow, 1973). However, several studies on their ultrastructure, cell wall chemistry, and molecular phylogeny, starting from the 1970s, have shown that the two groups are related and do not belong to the fungi (Perkins, 1972, 1973; Moss, 1985; Chamberlain and Moss, 1988).

Labyrinthulids are prevalent on or in living marine algae and sea grasses as parasites, commensals, or mutualists (Pokorny, 1967; Porter, 1990; den Hartog et al., 1996). Thraustochytrids, on the contrary, are rarely found on these living plants and appear to be inhibited by plant antimicrobials. Thraustochytrids are perhaps the only group of eukaryotes of an obligate marine occurrence that have an exclusively absorptive mode of nutrition. Thraustochytrids often abound on dead plant material such as macroalgae and probably play an important role as saprobes by virtue of extracellular enzyme production and chemical alteration of detritus (Raghukumar et al., 1994a, 1994b, 1995). Thraustochytrids rapidly colonize allochthonous material, such as submerged mangrove leaves (Findlay et al., 1986;

Raghukumar et al., 1995), and even swiftly settled within inorganic substrata placed in the sea (Raghukumar et al., 2000).

Labyrinthulomycetes reportedly cause diseases in animals, and there are indications that they live as commensals or mutualists within the guts and tissues of marine invertebrates, as well as being saprobic on such animal materials as feces and mollusk shells (Raghukumar, 2002). Thraustochytrids are common in the neritic and oceanic water column and sediments (Gaertner and Raghukumar, 1980; Raghukumar and Gaertner, 1980), including the deep sea (Raghukumar et al., 2001; Lõpez-Garcia et al., 2001).

Seasonal studies indicate poor correlation of thraustochytrid biomass with phytoplankton blooms, but often reveal it equivalent to that of bacteria during times of phytoplankton decay (Gaertner and Raghukumar 1980; Raghukumar and Gaertner, 1980; Raghukumar et al., 2001). Thraustochytrids were present in substantial numbers throughout the 150-m water column of the Arabian Sea during the end of the biologically productive summer and winter monsoons. Vertical distribution was seasonally variable (Raghukumar et al., 2001).

Labyrinthulomycetes may play an important role in mineralization of phyto- and zooplankton detritus in the sea. Their high content of ω-3 polyunsaturated fatty acids suggests that they may form an important link in the food web (Raghukumar, 2002).

4.9 FUNCTION OF FUNGI IN THE MARINE ENVIRONMENT

Marine fungi are major decomposers of woody and herbaceous substrates in marine ecosystems. The majority of described species may be isolated from wood and wood-related substrata (Figure 4.1), as they are able to aggressively degrade lignocellulose (Hyde et al., 1998a). Wood is a heterogeneous polymer made of polysaccharides, cellulose, and hemicellulose, with some lignin — a polyphenolic component (Fengel and Wegener, 1989). Fungal enzymes involved in wood degradation (cellulases and redox enzymes) were demonstrated in large number, and sometimes considerable amounts, in wood-inhabiting marine fungi. Beta-glucosidase and endoglucanase were the most frequent enzymes (80 to 100% of the strains) of cellulose metabolism. Many of the marine fungi showed high cellulase activity. The presence of laccase was different among the various fungal systematic groups, reaching its highest percentages in the basidiomycetes and ascomycetes, which mostly belong to the ecological groups of white-rot and soft-rot fungi, respectively (Rohrmann and Molitoris, 1992).

In saltmarshes, a principal role of aerobic filamentous fungi is the degradation of lignocellulose in vascular plants. Fungi are dominant secondary producers of the *S. alterniflora* decay system (Newell, 2001a), comprising up to 28% of living cordgrass standing crop (Newell and Porter, 2000). Ascomycetous fungi have been shown to be capable of degrading both the lignin and polysaccharide moieties of lignocellulose in the saltmarsh environment (Bergbauer and Newell, 1992; Newell, 1996). For example, most evidence suggests that fungi use nonspecific, extracellular enzymes to modify the lignin macromolecule (Gessner, 1980). Laccase belongs to a family of multicopper oxidases that are widespread in fungi. Laccases are one of the three families of enzymes that are considered responsible for the initial fragmentation of the lignin polymer and production of low-molecular-weight breakdown products. Enzymatic action removes various functional groups, side chains, and aromatic rings randomly from the lignin macromolecule (Kirk and Farrell, 1987).

A comparison of phylogenetic affiliation of amplified laccase genes was made with the fungal community based on internal transcribed spacer regions of rRNA (ITS) data associated with early and late decay stages of *S. alterniflora* blades (Buchan et al., 2002;

Lyons et al., 2003). Both the laccase and ITS libraries were dominated by sequences from three fungi: *Phaeosphaeria halima*, *Phaeosphaeria spartinicola*, and *Mycosphaerella* sp. 2 (Buchan et al., 2002; Lyons et al., 2003). The multiplicity of laccase types found within a single species possibly allows for broader substrate specificity and may confer an ecological advantage in the competition for space and nutrients in the decay system (Lyons et al., 2003).

Similar decomposition processes are common in mangrove habitats. Several genera of marine fungi isolated from decaying mangroves and sea grass were tested for the presence of laccase, an enzyme present in many genera of wood-rotting fungi. Xylanase and cellulase activities were observed in a majority of the fungi studied. The results indicate that laccase is widely distributed in fungi found on decaying lignocellulosic materials in the tropical marine environment and that a number of these fungi also contain other lignocellulose-modifying enzymes. Lignin peroxidase and manganese-dependent peroxidase appear to be relatively less common in these marine fungi (Raghukumar et al., 1994a, 1996). Marine fungi also displayed endoglucanase and cellobiohydrolase activity indicated by utilization of carboxymethylcellulose and crystalline cellulose, respectively. Variations in salinity appeared to have little effect on the extent of cellulolysis. Generally, peroxidase activity favored high salinities, whereas laccase was more pronounced at lower salinities (Pointing et al., 1998).

There is little data of *in situ* decomposition and nutrient cycling of mangrove by fungi. Ergosterol was measured in red mangrove (*Rhizophora mangle*) materials as an index of eumycotic fungal mass. The maximum value for standing crop of eumycotic fungal mass in submerged, decaying mangrove materials, calculated on the basis of the present ergosterol analyses, was 1.7% organic fungal mass of organic leaf mass. It may be that marine oomycotes (species of *Halophytophthora*), which do not contain ergosterol, are more important than ergosterol-containing fungi in the decay of submerged mangrove leaves (Newell and Fell, 1992).

Laminarin is a common polysaccharide in algae. Marine fungi are also capable of degrading laminarin and may have a role in the breakdown of seaweed. Laminarinase activity was associated mainly with the fungal mycelia. Quantitative growth studies of the fungus *Dendryphiella salina* showed that the mycelial activity was proportional to dry weight over the growth phase of the fungus and confirmed the low levels of extracellular activity (Grant and Rhodes, 1992).

4.10 SYMBIOTIC FUNGI IN THE MARINE ENVIRONMENT

Literature on fungal marine symbionts is scant. Mutualistic symbiotic associations of marine fungi occur with other organisms in three forms: lichens, mycophycobioses, and mycorrhizas.

4.10.1 Lichens

Lichen-forming fungi can occur on tidal coastal rocks where they can constitute major components of the biota. There are approximately 700 species known from coastal rocks (Hawksworth, 2000). The ecophysiology and biology of these fungi have been scarcely explored (Hawksworth, 2000). Most grow on rock surfaces in the intertidal zone and cannot survive constant immersion, although there are exceptions (Santesson, 1939). For instance, *Verrucaria serpuloides* has been recorded from a depth of 30 m (Lamb, 1973). Some species are only exposed at the lowest tides or when there are exceptional droughts. Lichens are vertically zoned in marine habitats. The heights of zones vary according to the degree of exposure and periods of inundation. The specialized lichens of the littoral

zone belong to the genera *Lichina* (2), *Pyrenocollema* (5), and *Verrucaria* (9) (Fletcher, 1973, 1975; McCarthy, 1991; Gilbert, 2001). Lichens are less well developed on coastal rocks in the tropics than in temperate regions. In some primitive lichens, the association between the fungal and algal components is loose. The algal component, which is usually microscopic, can be free living. *Chadefaudia corallinarum* can associate with the algae *Dermatoliton* and *Epilithon*, which grow as epiphytes on macroalgae. A few other genera of fungi, which form similar associations, are *Pyrenocollema*, *Stgmithum*, and *Turgidosculum* (Kohlmeyer and Kohlmeyer, 1979; Gilbert, 2001). As few as only 18 true marine lichen-forming fungi are described.

4.10.2 Mycorrhiza

There is an increased recognition today that mycorrhizas and other miscellaneous endophytic root fungus associations may substantially contribute to the life of not only individual plants but also whole communities, the nutrient balances of which, in all but fertile environments, are dependent on and integrated by the mycorrhizal fungi (Read, 1993). Their effect is highly relevant in abiotically stressed plant habitats.

It is interesting to note that saltmarsh plants were one of the earliest reported ecological plant groups showing mycorrhiza-like endophytes in roots (Mason, 1928). In spite of a small amount of evidence to the contrary (e.g., Peat and Fitter, 1993), pioneer saltmarsh plants and other obligate hydrohalophytes, belonging to several plant families, have often been reported as arbuscular mycorrhizal (AM) in tropical (or subtropical) and temperate situations. Halophytes from both coastal and inland Central European saltmarshes were examined for colonization by AM fungi and were found to be strongly colonized (Hildebrandt et al., 2001). The presence of mycorrhizas may enhance oxygen uptake and enhance stress resistance to salt in saltmarshes (Khan and Belik, 1995).

Recent studies noted AM associations in mangroves. There was nothing unique about the species composition and diversity of the AM fungi of the mangrove ecosystem, and only adaptive tolerance to salinity and inundation in the common AM fungi was indicated. Among the observed predominating AM fungal species, *Gigaspora margarita* is known for its submergence or high moisture tolerance (Khan, 1993). Dominant members of the mangrove plant community of the Ganges River estuary were all AM. Many of the known nonmycotrophic plant families also showed AM association, with intracellular hyphae and vesicles as the most discernible endophyte structures (Sengupta and Chaudhuri, 2002).

4.10.3 Mycophycobioses

Mutualistic symbiotic associations between marine fungi and macroalgae are called mycophycobioses. Such associations are obligate, and the habit of the alga predominates. The widely distributed brown seaweeds *Ascophyllum nodosum* and *Pelvetia canaliculata* are partners of *Mycophycias ascophylli*, and the red algae *Apophlaea lyallii* and *Apophlaea sinclairii*, endemic to New Zealand, are photobionts of *Mycophycias apophlaeae* (Kohlmeyer and Volkmann-Kohlmeyer, 1998). Members of the genus *Blodgettia* form mycophycobioses (Kohlmeyer and Kohlmeyer, 1979). The fungus *Turgidosculum complicatum*, which associates with the green algae *Praseola borealis* and *Praseola lessellata*, is thought to confer resistance to desiccation at low tides (Kohlmeyer and Kohlmeyer, 1979).

4.11 THE HUMAN EFFECT

Data for the effects of most types of disturbance on marine fungi are poor. Ascomycetous fungi are the principal drivers of the decomposition of shoots of smooth cordgrass (*S.*

alterniflora). Therefore, influences on saltmarsh ascomycetes by pollutants of saltmarshes could have far-reaching impacts. Examination of impacts of severe contamination of a Georgia saltmarsh by mercury and polychlorinated biphenyls (PCBs) revealed little or no influence of the toxicants on living standing crops (Wall et al., 2001) or sexual productivities of cordgrass ascomycetes (Newell and Wall, 1998). Extension of the examination of the saltmarsh/ascomycete response to sites containing other toxic pollutants (the chlorinated organocyclic insecticide toxaphene; chromium, copper, and lead; and polycyclic aromatic hydrocarbons [PAHs]) has shown that none of the additional toxicants engendered saltmarsh/fungal responses in the form of reduced living standing crops or sexual productivities. Thus, the ascomycetes of the cordgrass decay system appear to be as resistant to anthropogenic-pollutant poisoning as smooth cordgrass itself. Unless the fungal and plant resistance mechanisms involve degradation of the toxicants, this may imply that saltmarshes are especially dangerous as receiving sites for toxic waste because they may have the potential to readily move toxicants into the food web (Newell et al., 2000).

In mangrove habitats, studies vary in their deduction. There were significantly less diversity and numbers of fungi in an oil-polluted mangrove than in a healthy mangrove (Hyde, 1989b). The presence of hydrocarbons on substratum surfaces is known to reduce aeration and slow down fungal activity (Scherrer and Miller, 1989). Fungal diversity in the tropics appears to be related to plant diversity, and therefore, loss of the plant diversity is likely to result in loss of fungal diversity (Tsui et al., 1998). On the other hand, in the study of the intertidal fungi in Mauritius, the highest diversity (36 sp.) occurred at Beau Champ mangrove, an area contaminated with oil from an effluent of a sugar factory (Hyde et al., 1998b).

Disturbance, even in modest amounts, typically reduces biodiversity, but often without any documentable change in biomass or function (Howarth, 1991). Saprophytic fungi are involved in performing the ecosystem functions of decomposition and nutrient cycling within marine coastal habitats. Levin et al. (2001) hypothesized that species loss in highly redundant functional groups, such as fungi, where many species perform a similar function, and the loss of diversity as a result, will have limited effects on ecosystem function.

4.12 ESTIMATES OF MARINE FUNGI NUMBERS

There are approximately 80,000 species of described fungi (Kirk et al., 2001), representing only about 5% of the estimated 1.5 million species worldwide (Hawksworth, 1991). Proportionally, there are numerous undescribed species, and most habitats and hosts should provide a bounty of novel fungi that can be exploited in a wide variety of ways (Hyde, 2001). The extent of novelty discovered in recent monographic generic revisions and studies of species in particular habitats varies from 0 to 96%. Allowances for cryptic species, now known to be widespread by incompatibility and molecular studies, could justify an upward revision by a factor of at least five (Hawksworth, 2001).

The current estimates of total marine fungi comprise 1500 species (Jones and Mitchell, 1996). The number includes described species and incomplete or new species. This encompasses higher marine fungi (441 + 350) and lower fungi (100 + 32) such as trichomycetes (23), marine lichens (18 + 410), and thaustochytrides (40) that have been redescribed as pseudofungi. The numbers are referred to as indicative. It is likely to be a considerable underestimate of the biodiversity of this ecological group (Liberra and Lindequist, 1995; Clement et al., 1999).

Table 4.2 shows the number of higher fungi described in different periods. A rough calculation shows that 11 new species are added to the marine community per annum. If

Table 4.2 Number of Higher Marine Fungi Described in Different Periods

Division	Kohlmeyer and Kohlmeyer (1979)	Kohlmeyer and Volkmann-Kohlmeyer (1991)	Hyde and Pointing (2000)	Present Book
Ascomycota	149	255	360	453[a]
Basidiomycota	4	6	10	11
Anamorphic fungi	56	60	74	3
Total	209	321	444	467

[a] The high number is due to the changes in the systematic classification. This chapter follows the directions of Kirk et al. (2001, 2003).

calculations are based on the current estimate, the rate of discovered novelty species will not level out for another 30 years.

Fröhlich and Hyde (1999) propose that global estimates of fungal diversity require revision upwards. They base their statement on the fact that global estimates rely on a ratio of one plant host to six fungal species, based on temperate studies. The large numbers of fungi known to occur on *Nypa* palm, almost 40 intertidal species that are unique to *Nypa fruticans*, indicate a higher ratio. There are only two other species of mangrove palm that may support these intertidal palm fungi (*Calamus erinaceus* and *Oncosperma tigillarium*; Tomlinson, 1986); limited examination of decaying parts of these intertidal hosts have not revealed many similar fungi (Hyde and Alias, 2000). The upward revision based on the rate of host-specific intertidal fungi may also be applied to the estimates of marine fungi.

Only 5% of total estimated 1.5 million fungi species are known, of which higher fungi make up 97.5% of the known diversity. The question may be asked about the applicability of this ratio to marine fungi. A quick calculation may produce a rough assessment of a little under 10,000 species for marine fungi. We may consider revising down the number because the studies to date center on the diverse communities of coastal marine fungi. Nevertheless, the same consideration can be used to adjust up because of the many unresearched shores in tropical areas. The 1500 species estimate is based on described fungi and species that have been encountered but are new or have problematic descriptions. In their review of mangrove fungi, Sarma and Hyde (2001) state that most marine fungi have been described or at least collected. The assumption may be made that taking into account the current rate of discovery, the true diversity of marine fungi is much higher than the current estimate.

4.13 SHORT-TERM AND LONG-TERM GOALS OF MARINE FUNGI SCIENCE

As stated before, the characterization of any community can be achieved by describing three attributes: composition, structure, and function. The long-term goal facing researchers in the field of marine fungi is to be able to determine the three attributes for the different habitats harboring marine fungi. The documentation of marine fungi is still at the inventory stage, and many new taxa await discovery. Although inventory data are available for the marine fungi occurring on various substrata in several countries, there are still many regions and types of substrata that have not been investigated. The first goal of researchers is to

be able to compile local species richness lists for habitats such as the deep sea, European saltmarshes, and African mangroves, and for substrates such as living or dead coral and sea grasses, especially in the tropics.

One of the obstacles to the comprehension of the fungal aspect of the marine environment is entwined with the definition of marine fungi. For instance, many isolates from maritime substrates are considered terrestrial and not marine species. A recent study showed that marine fungal isolates, identified as terrestrial species, such as *Aspergillus* and *Trichoderma*, grew more abundantly as the seawater concentration increased. Even though the marine isolates were considered morphologically similar to terrestrial fungi, the two types were found to have different physiological characteristics (Masuma et al., 2001). Hyde et al. (2000) excludes fungi from extreme environments, such as the Dead Sea (Buchalo et al., 1998; Kis-Papo et al., 2001), pending the resolution of the question, What is a marine fungus? Yet isolates from the Dead Sea, considered terrestrial, were able to grow and survive in near-sea conditions (Kis-Papo et al., 2003a, 2003b), as are many of the terrestrial species recovered from marine locations. They may be considered facultatively marine. The debate on marine fungi criteria has not yet been concluded.

The second level of the community study after composition is structure, which includes the hierarchal configuration of species. As with many other organisms, only a few fungal species predominate on a given substratum or in a given habitat. Recent studies on intertidal mangrove fungi have provided information on frequency of occurrence, host and substratum specificity, colonization patterns, and seasonal occurrence (Sarma and Hyde, 2001). These studies centered on the Pacific and Indian Oceans. Little data, however, are available on the frequency of occurrence of mangrove fungi from the Atlantic Ocean. The eastern U.S. saltmarshes are well researched, but saltmarshes in Europe and other temperate areas have provided little or no knowledge as to the fungal diversity. The availability of fungal propagals and substratum colonization in the open sea is poorly researched. Many of the studies on the ecology of marine fungi have suffered from a lack of statistical analysis (Sarma and Hyde, 2001). The need for integrated research, with statistics on frequency of occurrence and spatiotemporal scales, is still extensive. The questions of the presence of key species and dominance order are unanswered, as are host and substratum specificity. Emphasis should also be given to the detailed study of the fungi on other intertidal plant hosts.

The part taken by fungi in ecosystem processes and their function mainly as decomposers of woody and herbaceous substrates is fundamentally understood, but an understanding of their relative importance and quantative data are scarce. The role of marine fungi in the decay of leaves and woody tissue requires further investigation, including interactions between fungi and invertebrates. There is little knowledge of the role of marine fungi in sediments and in the decay of dead animal parts. When fungi are isolated from marine sediments, typical fast-growing weedy species (e.g., *Aspergillus*, *Penicillium*) are usually recovered. It is unclear if these fungi are active in the sediments or if dormant spores are being isolated. Although fungi are also known to occur in the deep sea (Kohlmeyer and Kohlmeyer, 1979), we have little data of their role or importance in this ecosystem (Hyde et al., 1998a).

The last ecological arena is the consequences of human presence. Data on the effects of most types of disturbances on marine fungi are poor. Therefore, the need for organized studies investigating the effect of human disturbance on fungal diversity is critical. The various habitats should be investigated before irreversible changes occur. The marine environment is subject to increasing human disturbances such as industrial effluent discharge, sewage, oil spills, and leachates containing pesticides. Coastal and mangrove areas are most vulnerable when exposed to these stress factors.

Marine fungi may be encountered in diverse habitats encompassed in the marine environment. Although data have accumulated in the last half-dozen decades as this science has proliferated, for the present the gaps in our knowledge are almost the same scale as the knowledge acquired. The task remains for contemporary researchers to fill the geographical blanks and deepen the comprehension of the function and redundancy of marine fungal communities.

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5

Tropical Fungi

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5.1 INTRODUCTION

This chapter explores the distribution of fungal communities in tropical regions, noting the differences and similarities between groups of fungi that dominate selected habitats and substrates. The differences in tropical fungal communities and their special adaptations are highlighted and contrasted with those in temperate regions. The functional role of tropical fungi is also discussed and compared with those in other climatic zones. The hypothesis that fungi play a greater role throughout vertical strata of the forest than in other climatic zones is explored.

Our approach is to examine particular habitats in the tropics that have been relatively well investigated and for which data are available. Differences and similarities between groups of fungi that dominate different habitats are illustrated by examining graminicolous, folicolous, fruit- and seed-inhabiting, and freshwater fungi. The differences in tropical fungal communities and their special adaptations are illustrated using marine and mangrove fungi and freshwater lignicolous fungi, with a brief reference to palmicolous fungi. We examine the functional role by exemplifying the succession of saprobic fungi on leaves and how this may be related to resident endophyte communities vs. chance saprobes. Finally, we explore the hypothesis that fungi play a greater role throughout the vertical strata in the forest by exploring mangrove fungi, intertidal grass fungi, bamboo fungi, and folicolous fungi in forests.

5.1.1 Definition and Distribution of Fungal Communities

The definition and distribution of fungal communities are provided elsewhere in this book (see Chapters 2); these definitions also apply to the tropics.

5.2 DIFFERENCES IN FUNGAL NUMBERS AND COMPOSITION OF COMMUNITIES

If greater plant diversity results in greater fungal diversity, then fungal communities in the tropics are probably more highly diverse than those in temperate regions. Microfungi in the tropics are relatively unknown, yet probably constitute the majority of fungi (Rossman, 1997). Despite the diversity of habitats within the tropics, Samuels and Rossman (1992) claimed that few fungi are strictly tropical. Several recent studies, however, have shown that there is certainly a difference in fungal communities between the tropics and temperate regions (e.g., Fröhlich and Hyde, 2000; Taylor and Hyde, 2003). We, therefore, choose four relatively well-studied substrata to explore these issues. We also return to the issue of fungal numbers later in this chapter when we discuss host specificity, recurrence, and exclusivity.

5.2.1 Graminicolous Fungi

One of the major terrestrial biomes from which large numbers of fungi are known is the grasslands (Farr et al., 1989). These harbor a diverse range of grass taxa and have a worldwide distribution. There are differences and similarities in fungal communities on grasses in tropical and temperate regions, and examples are discussed below.

More than 4000 new fungal taxa had been described as being associated with grasses (Cannon and Hawksworth, 1995). Yet knowledge of fungal diversity on grasses in the tropics is essentially poor. Most of our knowledge of fungal communities on grasses is based on studies in temperate regions. *Lophodermium* species (particularly Rhytismatales) are well characterized from 13 Ukrainian grass species (Minter and Dudka, 1996). *Lophodermium arundinaceum* has been found on dead stems of *Phragmites australis* in Ukraine. However, members of this genus were unrecorded on tropical grasses in the study of Wong and Hyde (2001).

The major study dealing with fungal diversity on the Gramineae in the tropics (Hong Kong) is that of Wong and Hyde (2001). Senescent culms of *Panicum maximum, Pennisetum purpureum, Phragmites australis (freshwater), Miscanthus floridulus, Saccharum arundinaceum,* and *Thysanolaena maxima* were collected over a 2-year period from different regions in Hong Kong and examined for fungi (Table 5.1). The common fungi on tropical grasses include ascomycetes and their anamorphs, smuts, and rusts (Wong and Hyde, 2001; Shivas and Vánky, 2003). There have been a greater number of studies of fungi on grasses in temperate regions (e.g., Ellis and Ellis, 1985; Farr et al., 1989; Dix and Webster, 1995). Ellis and Ellis (1985) illustrated the plurivorous taxa on grasses in the U.K., which include discomycetes (*Belonium*, *Calycella*, *Crocicreas*, *Dasyscyphus*, *Hymenoscyphus*, *Lophodermium*, *Micropeziza*, *Mollisia*, *Pezizella*), ascomycetes (see Table 5.1), anamorphic fungi (see Table 5.1), smuts (*Urocystis*, *Ustilago*), and rusts (*Puccinia*, *Uromyces*).

Although comparison of these data sets provides a crude collation, the contrast is worthwhile and revealing. In the U.K., plurivorous genera overlap, but most species differ from those recorded in Hong Kong. *Chaetomium* and *Mycosphaerella* species, plurivorous on grasses in the U.K., were not recorded in Hong Kong, and *Pleospora* were more common on grasses in the U.K. Several other species (e.g., *Botryosphaeria festucae*, *Monographella nivalis*) were only recorded in the U.K. Conversely, common genera in Hong Kong (e.g., *Diaporthe*, *Ophioceras*, *Phragmitensis*) were not regarded as plurivorous in the U.K., while several other species (e.g., *Annulatascus triseptatus*, *Linocarpon angustatum*) are absent in the U.K. A small number of species (e.g., *Alternaria alternata*, *Tetraploa aristata*) are common on grasses in both climatic regions. The inference is that

Table 5.1 Common Fungi on Grasses in Temperate U.K. and Tropical Hong Kong

U.K.		Hong Kong	
Ascomycetes	**Anamorphic Fungi**	**Ascomycetes**	**Anamorphic fungi**
Acrospermum graminum	*Actinothyrium graminis*	*Annulatascus triseptatus*	*Alternaria alternata*
Botryosphaeria festucae	*Alternaria* spp.	*Didymosphaeria conoidea*	*Cladosporium cladosporioides*
Chaetomium elatum	*Arthrinium* spp.	*Diaporthe* spp.	*Colletotrichum* spp.
Claviceps purpurea	*Ascochyta* spp.	*Leptosphaeria* spp.	*Fusarium* spp.
Creopus spinulosus	*Cercosporidium graminis*	*Linocarpon angustatum*	*Nigrospora* state of *Khuskia oryzae*
Epichloe typhina	*Colletotrichum graminicola*	*Lophiostoma* spp.	*Periconia* spp.
Erisyphe graminis	*Dictyosporium elegans*	*Macrospora scirpicola*	*Petrakia paracochinensis*
Gaeumannomyces graminis	*Dilophospora* state	*Niptera excelsior*	*Phaeoisaria* spp.
Gibberella zeae	*Dinemasporium* sp.	*Ornatispora taiwanensis*	*Phoma* spp.
Keissleriella culmifida	*Drechslera* spp.	*Ophioceras* spp.	*Phomopsis* spp.
Leptosphaeria spp.	*Epicoccum purpurascens*	*Paraphaeosphaeria schoenoplecti*	*Septoria* spp.
Lophiostoma semiliberum	*Fusarium* spp.	*Phaeosphaeria variiseptata*	*Sporidesmium* spp.
Monographella nivalis	*Hendersonia* sp.	*Phomatospora* spp.	*Stachybotrys kampalensis*
Mycosphaerella spp.	*Myrothecium* spp.	*Phragmitensis* spp.	*Tetraploa aristata*
Ophiosphaerella herpotricha	*Periconia* spp.	*Pleospora penicillus*	
Paradidymella holci	*Pithomyces chartarum*		
Paraphaeosphaeria michotii	*Pseudoseptoria* spp.		
Phomatospora spp.	*Pseudocercosporella herpotrichoides*		
Phyllachora graminis	*Rhynchosporium* spp.		
Pleospora spp.	*Rotula graminis*		
Pyrenophora trichostoma	*Septoria* sp.		
Trichothyrina sp.	*Stagonospora subseriata*		
Tubeufia helicomyces	*Tetraploa aristata*		
	Torula herbarum		
	Ulocladium spp.		

Adapted from Ellis and Ellis, *Microfungi on Land Plants*, Croom Helm, London, U.K., 1985; Wong and Hyde, *Mycol. Res.*, 105, 1485–1491, 2001.

besides some overlapping species, the fungi on grasses in tropical Hong Kong and temperate U.K. generally differ.

One common grass reed species, *Phragmites australis*, has received considerable attention by researchers (e.g., Farr et al., 1989; Poon and Hyde, 1998a; Wong and Hyde, 2001). Some common genera frequently recorded on *P. australis* from tropical and temperate regions are listed in Table 5.2. Species of *Anthostomella*, *Chaetomium*, *Fusarium*, *Leptosphaeria*, *Massarina*, *Ophiobolus*, *Phaeosphaeria*, *Phoma*, *Phomatospora*, and *Stagonospora* appear to have a wide distribution and are commonly found in both tropical and temperate areas. On the other hand, *Cladosporium*, *Cytoplea*, *Dinemasporium*, *Frondisphaeria*, *Gaeumannomyces*, and *Pleurophragmium* appear to occur only on tropical *P. australis*. Poon and Hyde (1998a) assessed diversity of the fungal mycota found on decaying stems and leaf sheaths of *P. australis* in two different estuarine habitats. In both habitats, frequently occurring taxa were *Aniptodera phragmiticola*, *Cladosporium* sp., *Colletotrichum* sp., *Fusarium* sp., *Lignicola laevis*, *Phomopsis* sp., and *Trichoderma* sp.

5.2.2 Folicolous Fungi

One might suspect that greater numbers of phylloplane fungi may occur in the tropics. Certainly there are a large number of sooty molds and related taxa, but most of these are described based on host occurrence and there is little evidence to indicate that these taxa are truly host specific. Lee and Hyde (2002) found very few truly phylloplane fungi in mangroves of Hong Kong, and if this applies to rain forests, this would affect estimates of fungal numbers greatly.

It is difficult to compare the fungal communities on leaves in the tropics with those in temperate regions because fungal presence is related to host (Hyde et al., in press; Paulus et al., in press) and a particular host species does not naturally grow in both the tropics and temperate regions. We can, however, compare fungal communities on nonspecific leaf litter in tropical vs. temperate regions.

Most studies of fungi on leaf litter have been carried out by isolations from leaf washings (Paulus et al., 2003). We do not consider this representative of the fungi involved in litter decay, as the ability to grow on selective agar is likely to influence results. Direct observation of leaves following incubation in damp chambers is therefore preferred and is compared here. A study of litter fungi in an Oakwood forest in the Lake District of the U.K., using direct observation, was carried out by Hering (1965). The dominant taxa were anamorphic taxa, including *Cladosporium herbarum*, *Paecilomyces farinosus*, *Verticillium lecanii*, *Gliomastix murorum*, *Trichoderma viride*, and *Mucor hiemalis* (Table 5.3). Parungao et al. (2002) investigated fungi on litter of 13 tree species in two tropical rain forests in North Queensland, Australia, by direct observation and recorded 57 microfungi, most of which were anamorphic taxa. The most dominant taxa were *Chaetospermum artocarpi*, *Dictyochaeta* sp. 2, *Lanceispora* sp., *Ophioceras fusiforme*, and *Beltraniella* sp. at Butchers Creek and *Dictyochaeta* sp. 2 and 3, Hyphomycete sp. 1, *Beltrania rhombica*, *Dictyochaeta zeylanicum*, and *Lophodermium* sp. at Mt. Lewis (Table 5.3). In a study of fungi on *Manglietia garrettii* litter by direct observation in a tropical forest in northern Thailand, Promputtha et al. (2002) found 37 taxa comprising 20 ascomycetes and 17 anamorphic fungi. Dominant taxa were *Sporidesmium crassisporum*, *Hyponectria* sp. nov., *Gliocladium* sp. 1, *Cylindrocladium floridanum*, *Lasiosphaeria* sp., and *Pseudohalonectria suthepensis* (Table 5.2). There was no overlap between the fungi decaying tropical and temperate litter, indicating that different fungal communities operate in the different climatic regions. Although hyphomycetes were generally more common, ascomycetes were also involved in the decay of leaves in the tropics but were notably absent in temperate regions. This may be a reflection of the size of leaves studied, leaf composition, leaf nutrient content,

Table 5.2 Common Genera Found So Far on *Phragmites australis*

Tropical Regions	References	Temperate Regions	References
Aniptodera (3)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Anthostomella**	Shearer (1993)
Acremonium (2)	Poon and Hyde (1998a), Lu et al. (2000)	*Belainopsis*	Shearer (1993)
*Anthostomella**	Wong and Hyde (2001), Lu et al. (2000)	*Cercosporidium*	Farr et al. (1989)
Arthrinium (2)	Poon and Hyde (1998a)	*Ceriosporopsis*	Shearer (1993)
*Chaetomium**	Poon and Hyde (1998a)	*Chaetomium** (2)	Shearer (1993)
Cladosporium	Poon and Hyde (1998a), Wong and Hyde (2001)	*Claviceps*	Farr et al. (1989)
Colletotrichum (6)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Dasyscyphus* (2)	Shearer (1993)
Cytoplea	Poon and Hyde (1998a)	*Deightoniella*	Farr et al. (1989)
Dictyochaeta	Wong and Hyde (2001)	*Didymella*	Shearer (1993)
Dinemasporium	Poon and Hyde (1998a), Wong and Hyde (2001)	*Fusarium**	Farr et al. (1989)
Frondisphaeria	Wong and Hyde (2001)	*Graphyllium*	Farr et al. (1989)
*Fusarium** (3)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Helotium* (2)	Shearer (1993)
Gaeumannomyces	Poon and Hyde (1998a)	*Hendersonia*	Farr et al. (1989)
Gliomastix (2)	Poon and Hyde (1998a)	*Lasiosphaeria*	Shearer (1993)
Halosarpheia (3)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Leptosphaeria**	Shearer (1993)
		Lophiostoma	Shearer (1993), Farr et al. (1989)
Helminthosporium	Farr et al. (1989)	*Lophodermium*	Minter and Dudka (1996)
Kylindria	Wong and Hyde (2001)	*Massarina**	Shearer (1993)
*Leptosphaeria** (3)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Micronectriella*	Shearer (1993)
Lignincola	Poon and Hyde (1998a)	*Mollisia*	Shearer (1993)
*Massarina** (2)	Poon and Hyde (1998a)	*Mycosphaerella*	Farr et al. (1989)
Microsphaeropsis (4)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Ophiobolus**	Shearer (1993)
Nectria	Poon and Hyde (1998a)	*Passeriniella*	Shearer (1993)
Neottiosporina (4)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Pezizella*	Shearer (1993)
*Ophiobolus** (4)	Wong and Hyde (2001)	*Phaeosphaeria** (2)	Shearer (1993), Farr et al. (1989)
Ophioceras	Wong and Hyde (2001)	*Phoma**	Farr et al. (1989)

Table 5.2 Common Genera Found So Far on *Phragmites australis* (Continued)

Tropical Regions	References	Temperate Regions	References
Penicillium	Poon and Hyde (1998a)	*Phomatospora**	Shearer (1993)
Pestalotiopsis (3)	Poon and Hyde (1998a), Wong and Hyde (2001), Lu et al. (2000)	*Phragmopeltis*	Farr et al. (1989)
Phaeoisaria (2)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Pseudographis*	Farr et al. (1989)
*Phoma** (8)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Pseudoseptoria*	Farr et al. (1989)
*Phomatospora** (2)	Wong and Hyde (2001)	*Pythium*	Farr et al. (1989)
Phomopsis (12)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Scolicotrichum*	Farr et al. (1989)
Phragmitensis (3)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Stagonospora**	Farr et al. (1989)
Pleospora (2)	Poon and Hyde (1998a), Wong and Hyde (2001)	*Thielavia*	Farr et al. (1989)
Pleurophragmium	Wong and Hyde (2001)	*Vibrissea*	Shearer (1993)
Pragmopycnis (2)	Wong and Hyde (2001)		
Pseudohalonectria	Poon and Hyde (1998a)		
Pseudorobillarda	Poon and Hyde (1998a)		
Rhinocladiella (10)	Wong and Hyde (2001)		
Sarocladium (3)	Poon and Hyde (1998a), Wong and Hyde (2001)		
Sclerostagonospora	Poon and Hyde (1998a)		
Septoria (7)	Wong and Hyde (2001)		
Septoriella	Poon and Hyde (1998a)		
Sporidesmium (3)	Wong and Hyde (2001)		
Stachybotrys	Poon and Hyde (1998a)		
*Stagonospora**	Poon and Hyde (1998a)		
Tetraploa (2)	Poon and Hyde (1998a), Wong and Hyde (2001)		
Trichoderma	Poon and Hyde (1998a)		
Zopfiella	Poon and Hyde (1998a)		

Note: Numbers in parentheses represent the number of different species collected, and asterisks represent common genera found in both tropical and temperate regions.

Table 5.3 Dominant Taxa on Decaying Litter in Tropical and Temperate Habitats Identified after Damp-Chamber Incubation

Temperate Mixed Litter in Lake District	Tropical Mixed Litter at Butchers Creek[a]	Tropical Mixed Litter at Mt. Lewis[a]	Tropical *Magnolia lilliputi* Leaves in Chiang Mai[a]
Cladosporium herbarum	*Chaetospermum artocarpi*	*Dictyochaeta* sp. 2	*Sporidesmium crassisporum*
Paecilomyces farinosus	*Dictyochaeta* sp. 2	*Dictyochaeta* sp. 3	*Hyponectria* sp. nov. 1
Verticillium lecanii	*Lanceispora* sp. 3	Hyphomycete sp. 1	*Gliocladium* sp. 1
Gliomastix murorum	*Ophioceras fusiforme*	*Beltrania rhombica*	*Cylindrocladium floridanum*
Trichoderma viride	*Beltraniella* sp.	*Dictyochaeta zeylanicum*	*Lasiosphaeria* sp.
Mucor hiemalis	*Dictyochaeta hughesii*	*Lophiodermum* sp.	*Pseudohalonectria suthepensis*
Epicoccum nigrum	*Dictyochaeta zeylanicum*	*Dictyochaeta* sp. 1	*Hypoxylon* sp.
Aureobasidium pullulans	*Guignardia* sp. 1	*Ellisiopsis gallesiae*	*Bionectria ochroleuca*
Cephalosporium spp.	*Helicosporium decumbens*	*Gliocladium cylindrospermum*	*Dokmaia monthadangii*
Cylindrocarpon radicicola	*Lanceispora* sp. 2	*Lasiosphaeria* sp.	*Volutella* sp.
	Helicosporium sp.		

[a] Dominant by percentage occurrence.

Adapted from Hering, *Trans. Br. Mycol. Soc.*, 48, 391–408, 1965; Parungao et al., *Biodiversity Conserv.*, 11, 1185–1194, 2002; Promputtha et al., *Fungal Diversity*, 10, 89–100, 2002.

pollution, or some other unknown factors. Numerous ascomycetes (e.g., Gnomoniaceae, *Hyponectria*, and *Pseudomassaria* spp.) are, however, known from temperate leaf litter (Barr, 1964, 1977, 1978; Ellis and Ellis, 1985), but were not recorded in the succession study of Hering (1965).

5.2.3 Fruit- and Seed-Inhabiting Fungi

Our knowledge of the taxonomy, ecology, and distribution of any fungi occurring on wild fruits and seeds is inadequate in both temperate and tropical regions. It is therefore difficult to draw definite conclusions as to whether some of the known fungi are restricted to particular hosts and geography. Although numerous fruit- and seed-inhabiting fungal taxa occur in the tropics, most is known about postharvest fungi that cause economically important loss to cereals and edible fruits (Tang et al., 2003). A comprehensive survey of fungi associated with wild fruits in Hong Kong was carried out by Tang et al. (2003), while Somrithipol et al. (2002) surveyed fungi on rain forest seeds. Tang et al. collected mature wild fruits from 18 native plant species in Hong Kong to investigate types of fungi more likely to be involved in fruit decay. Five hundred and forty fruit samples examined yielded 595 identifications belonging to 101 taxa. Most were anamorphic taxa (79%), while ascomycetes accounted for only 18%. *Colletotrichum* (mainly *C. gloeosporioides*) and *Phomopsis* (mainly *P. archeri*) were the most widespread genera. Other frequently collected anamorphic genera included *Cladosporium*, *Fusarium*, *Pestalotiopsis*, and *Phoma*. Common ascomycetous genera were *Glomerella*, *Guignardia*, and *Massarina*. Tang et al. (2003) compared their findings with previous studies on cultivated fruits and found that fungi involved in decay on wild fruits are different from those on cultivated fruits. The fungi on wild fruits in temperate regions are less well studied but may also yield unique species.

Somrithipol et al. (2002) studied 130 mature pods of *Delonix regia* in a succession study. *Aspergillus*, *Chaetomium*, *Penicillium*, and *Rhizopus* species were dominant on samples collected directly from the tree. Surprisingly, similar fungi (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., and *Rhizopus* sp.) were found to be involved in the decay of breadfruit (*Artocarpus communis*), which was kept in storage (Amusa et al., 2002). A total of 70 fungi were recorded from pods of *D. regia* (50 anamorphic fungi and 12 ascomycetes), with *Cylindrocladium* sp., *Dictyochaeta* sp., *Phaeoisaria clematidis*, *Phoma* sp., and *Sporoschisma* spp. occurring at a high frequency during the 5-month succession study. Most of the fungi recorded from wild fruits by Tang et al. (2003) were different from those identified on seeds by Somrithipol et al. (2002).

There currently appear to be little data available regarding fungal colonization by saprobes on wild fruits in temperate regions. Some studies on seed- and fruit-inhabiting fungi were carried out in the temperate areas of the southern and northern U.S., but only *Xylaria* species were collected. Rogers (1978) isolated a new species, *Xylaria magnoliae*, which was found to be a common saprobe on decaying aggregate fruits of *Magnolia grandiflora*. Rogers also discussed the morphological affinities of *X. magnoliae* with other *Xylaria* species isolated from fruits of different host species. *X. magnoliae* and *X. carpohila* were considered to be predominantly temperate species, while *X. culleniae* and *X. ianthinovelutina* have been found to occur mostly on leguminous fruits in tropical regions (Martin, 1970). With intensive collections from American tropics and subtropics, however, Gonzalez and Rogers (1989) and Laessøe and Lodge (1994) recorded a number of *Xylaria* species from fruits (including *X. magnoliae* from *Magnolia schiedeana*), and they mentioned that the same species tended to colonize the same host genus, i.e., was host specific.

There is higher fruit diversity in the tropics than in temperate regions, and hence the range of resources or substrates available for fungal utilization is greater. However, it is not clear whether there are unique fungal communities associated with different fruit.

Tang et al. (2003) suggested that fungal communities are more likely correlated with nutrients, antimicrobial compounds, and the physical structure of the fruits. They found that persistent fruits, such as *Ardisia* spp., *Dichroa febrifuga*, *Sarcandra glabra*, and *Wikstroemia nutans*, have very low diversity and different assemblages compared with nonpersistent ones (Tang et al., in press).

5.2.4 Freshwater Fungi

A broad definition of freshwater fungi includes fungi that function entirely or partly in freshwater (Goh and Hyde, 1996; Thomas, 1996). In this chapter, we refer to freshwater fungi whose habitat may be of an aquatic nature or fungi that colonize submerged organic materials. Ascomycetes, Ingoldian fungi, and non-Ingoldian hyphomycetes are common in freshwater and are therefore discussed here.

Most investigations of freshwater fungi had previously been carried out in temperate regions, such as the U.K. and U.S. However, several recent studies have provided data for the tropics. By 2003, there were 177 freshwater ascomycetes recorded from the tropics, representing 35% of the 511 ascomycetes species recorded worldwide (Cai et al., 2003a).

5.2.4.1 Characters of Freshwater Fungal Communities in the Tropics

Freshwater Ascomycetes. Hyde et al. (1997) stated that most of the ascomycetes that they had encountered in the tropics were new species from other climatic zones that had not been reported. They therefore suggested that a distinct tropical freshwater ascomycete community existed. Of the 511 freshwater ascomycetes listed by Cai et al. (2003a), 177 species are reported from the tropics, 354 from the temperate regions, and only 20 overlap. This indicates that different ascomycete communities occur in tropical vs. temperate regions. This is also supported by the absence of discomycetes in the tropics. Only 4 of 110 known freshwater discomycetes are reported from the tropics (Cai et al., 2003a). One reason may be that most freshwater discomycetes have been reported from herbaceous macrophytes (in particular grasses) and macrophytes that have not been extensively studied in the tropics (Shearer, 2001). However, the studies of Wong and Hyde (2001, 2002) on submerged grasses in Hong Kong support the idea that fungal communities in the tropics differ, as they identified no discomycetes.

Annulatascus-like species are commonly encountered freshwater ascomycetes in the tropics, but they are less well reported from temperate regions (Hyde et al., 1997; Shearer, 2001; Cai et al., 2003a). This group of fungi have unitunicate cylindrical asci with a relatively massive refractive apical apparatus and ascospores with various types of appendages or sheaths (Wong, M.K.M. et al., 1998; Wong, S.W. et al., 1998). Other common tropical freshwater fungi include species from *Aniptodera*, *Jahnula*, *Massarina*, *Ophioceras*, *Pseudohalonectria*, and *Savoryella* (Goh and Hyde, 1996). These genera are also found in temperate regions, but the species mainly differ (e.g., River Coln, U.K., Hyde and Goh, 1999; Liput River, Philippines, Cai et al., 2003b).

Ingoldian Fungi. Ingoldian fungi grow on submerged leaves and twigs but are relatively sparse on woody substrates (Willough and Archer, 1973; Goh, 1997). They abound in fast-flowing streams and well-aerated lakes. Ingoldian fungi actively grow and sporulate underwater and are typical freshwater fungi. They predominantly comprise conidia with two basic shapes: sigmoid and branched. These conidial shapes aid in the dispersal. Iqbal and Webster (1973) found that air bubbles readily trap these conidia and bring them to the surface. The Ingoldian fungi have been more intensively studied than the freshwater ascomycetes (Shearer, 1993). Wood-Eggenschwiler and Bärlocher (1985) concluded that temperature and riparian vegetation are the main factors in determining

Ingoldian fungal communities. In the tropics, both temperature and riparian vegetation are distinctly different from that in temperate regions, and this may result in different fungal communities. Wood-Eggenschwiler and Bärlocher (1985) concluded that the distribution of Ingoldian fungi is either cosmopolitan, restricted to pantemperate or pantropical regions, or, in a few cases, have a restricted distribution.

There is limited data on Ingoldian fungi in the tropics, although recent studies include those of Betancourt and Justiniano (1989), who reported 35 species from Puerto Rico, and Justiniano and Betancourt (1989), who recorded 20 species in Puerto Rico. Recent studies of Ingoldian fungi in the tropics are those of Chan et al. (2000a, 2000b) from Hong Kong. They reported 41 species from 26 different genera.

Non-Ingoldian Freshwater Hyphomycetes. Non-Ingoldian freshwater hyphomycetes include aero-aquatic fungi and the lignicolous aquatic fungi. Aero-aquatic fungi are usually found on decaying plant materials in slow-flowing streams, stagnant ponds, and ditches (Goh and Hyde, 1996). They sporulate only when the substrate is exposed to air, forming buoyant propagules capable of dispersal when the substrate is submerged (Webster and Descals, 1981). The lignicolous aquatic hyphomycetes are those saprobic fungi growing on organic substrates under water, mostly woody materials of deciduous plants. When the substrates are, for various reasons, no longer submerged, they produce conidia that are capable of dispersal in air and water.

Goh (1997) reviewed the tropical freshwater hyphomycetes and recorded approximately 280 species in 143 genera (including Ingoldian fungi) from the tropics. Among these fungi, 26 species are regarded as "apparently tropical" species, while there are 19 species regarded as cosmopolitan. The diversity of tropical freshwater hyphomycetes was shown to overlap with those in temperate regions. Kuthubutheen (1993) also commented that many taxa in freshwater habitats in the tropics are either cosmopolitan or apparently tropical.

5.4.2.2 Is the Biodiversity Higher in the Tropics?

Most freshwater fungi have been reported from the temperate regions. This higher biodiversity in the temperate regions is, however, thought to reflect greater collection efforts (Wood-Eggenschwiler and Bärlocher, 1985; Shearer, 2001). Wood-Eggenschwiler and Bärlocher suggested that the freshwater ecosystems in the tropics are unique, while those in temperate regions provide more niches.

A direct comparison of the freshwater fungal diversity between tropical and temperate streams is difficult because factors such as riparian vegetation, pollution, and water chemistry may affect fungal communities. Investigations of fungi on substrates in Lake Barrine, North Queensland, a tropical region, yielded 1.4 species per sample (Hyde and Goh, 1998); the Plover Cove Reservoir, Hong Kong, a subtropical reservoir, yielded 2.4 species per sample (Goh and Hyde, 1999); and Lake Fuxian, a temperate lake in China, yielded 1.6 species per sample (Cai et al., 2002), indicating that the greatest diversity is in lakes in subtropical regions. Only 0.86 taxa per sample were identified in the River Coln, Britain (Hyde and Goh, 1999), whereas 2.28 taxa per sample were identified in the Liput River in the Philippines (Cai et al., 2003b), indicating that tropical streams support a higher diversity.

5.3 TROPICAL FUNGAL COMMUNITIES AND THEIR SPECIAL ADAPTATIONS

Many genera have representative species in both the tropics and subtropics, although the species found in either region may differ. In this section we explore whether fungi in

the tropics have special adaptations compared with those in temperate regions. Two habitats, marine and mangrove fungi and freshwater fungi, are discussed. Tropical fungi may have variously adapted ascospores, often with appendages or sheaths (sometimes elaborate), which are believed to aid in dispersal (Hyde and Jones, 1989b). This is particularly well illustrated in the palm fungi (see Fröhlich and Hyde, 2000; Hyde et al., 2000; Taylor and Hyde, 2003). A glance through *Microfungi on Land Plants* by Ellis and Ellis (1985) on fungi on British plants indicates that far fewer temperate fungi have ascospores with similar adaptations.

5.3.1 Marine and Mangrove Fungi

Tropical and temperate marine fungi are adapted for life in the sea; however, there is very little overlap between those species found in tropical areas and those of temperate regions (Kohlmeyer and Kohlmeyer, 1979). Members of the Halosphaeriales, in particular, have soft-walled ascomata, early deliquescing asci, and ascospores with large lipid globules, often with elaborate appendages (Hyde and Pointing, 2000). The appendages of tropical marine fungi are, however, no more elaborate than their temperate counterparts (Hyde and Sarma, 2000).

The same habitat groups of marine fungi occur in the tropics (e.g., arenicolous, growing in association with sand; algicolous, growing in algae) as in temperate regions, although species composition may differ (Hyde and Jones, 1989a). Habitat groups unique to the tropics are manglicolous fungi, those growing in mangroves, and coral-inhabiting fungi (Kohlmeyer and Kohlmeyer, 1979). Mangroves are the tropical equivalent of salt marshes, and the fungi on mangroves are unique to this habitat (Kohlmeyer and Kohlmeyer, 1979; Hyde and Jones, 1988; Sarma and Hyde, 2001). These fungi have the ability to grow on substrates that are intermittently submerged in seawater where the salinity may range from close to freshwater to higher than normal seawater. For instance, the fungi occurring on *Nypa* palm in tropical Asian mangroves often grow in freshwater or water that is slightly saline (Hyde, 1992). More than 40 species are unique to this habitat, i.e., palm substrata intermittently submerged in the sea (Hyde and Alias, 2000). The fungi occurring in this habitat include unique species belonging to typical palm genera (e.g., *Oxydothis nypae*, *Linocarpon appendiculatum*), unique species belonging to typical marine genera (e.g., *Helicascus nypae*), unique genera (*Frondicola*), and common marine species (*Lignincola laevis*, *Lulworthia grandispora*). Some species have interesting adaptations that are probably related to dispersal. *Carinispora nypae* has an elaborate sheath that has the shape of keel, while in *Frondicola tunitricuspis* the sheath is drawn out in four directions (Hyde, 1992). *Linocarpon* species have polar appendages that are variously complex when viewed under the transmission electron microscopy (TEM) (Yanna et al., 2004).

In the rear areas of some mangroves salinities are much higher than 35‰, which is the salinity of oceanic seawater. The salinity depends on the extent of rainfall and is often seasonal. Because of the high salinities, the tree species at the rear of mangroves are stunted, and in places, the mangrove trees are replaced by a few halotolerant plant species (e.g., *Ceriops tagal*, *Halosarciea halocnemoides*). Fungi in this region are few, but species such as *Cryptovalsa halosarceiicola* and *Pyrenographa xylographoides* are able to tolerate these extreme conditions and are probably involved in the decay of dead materials (Hyde, 1993; Alias et al., 1996).

5.3.2 Freshwater Lignicolous Fungi

Dispersal and attachment of propagules is a problem faced in the life cycle of freshwater fungi. Mechanisms of fungal adhesion are reviewed by Jones (1994), while the dispersal

of freshwater fungi has been addressed by Hyde and Goh (2003). Many species are clearly adapted to life in freshwater, as their propagules have specialized aquatic dispersal abilities.

5.3.2.1 Special Adaptations of Freshwater Fungi in the Tropics

Freshwater Ascomycetes. Tropical freshwater fungi often have more elaborate adaptations for dispersal in freshwater habitats than their temperate counterparts (Hyde and Goh, 2003). Species of *Lophiostoma* and *Massarina* are commonly collected in freshwater habitats, and ascospores often have sheaths for dispersal and attachment (Hyde and Goh, 2003). Sheaths in the tropics, however, may be more elaborate. In the tropical species *Fluviatispora reticulata*, the ascospore wall is echinate, having radiating striations arising from the spore wall and a spreading mucilaginous sheath becoming irregular in appearance with age (Hyde, 1994). Hamate or cap-like appendages, which uncoil to form viscous threads, are a common adaptation of freshwater fungi that appear widely in both temperate regions and the tropics. Freshwater species of *Aniptodera*, *Halosarpheia*, and *Phaeonectriella* have hamate or cap-like appendages that are initially held tight against the ascospore wall (Shearer, 1989; Hyde et al., 1999a). Filamentous ascospore appendages are commonly produced in several genera in the Annulatascaceae that are mostly found in tropical streams. In *Cateractispora bipolaris* the unicellular ascospores are provided with polar pad-like appendages that eventually spread in water to form long drawn-out strands (Hyde et al., 1999b). In *Diluviocola capensis* the unicellular ascospores have a cone-shaped hood that unravels to form filaments (Hyde et al., 1998b).

Ingoldian Hyphomycetes. It has been suggested that there are several factors that contribute to the success of these aquatic hyphomycetes (Webster and Descals, 1981; Read et al., 1992; Hyde and Goh, 2003). In particular, their unique conidial forms, invariably two- to several-armed or sigmoid shaped, have been shown to aid in their dispersal and subsequent attachment (Iqbal, 1995). Both tropical and temperate species are adapted in this way.

Non-Ingoldian Hyphomycetes. Long mononematous stipitate conidiophores, standing erectly on the substrata and bearing masses of conidia at their apex, are characteristic of most of the non-Ingoldian hyphomycetes (Kuthubutheen, 1993). Examples are found in species of *Acrogenospora*, *Cryptophiale*, *Dictyochaeta*, *Monotosporella*, *Pleurophragmium*, *Spadicoides*, and *Thysanophora* (Kuthubutheen, 1993; Goh, 1997). Some fungi, however, have erect synnemata with several to many conidiophores compacted together, such as in species of *Didymostilbe* and *Phaeoisaria* (Ellis, 1971). The ecological role of the long erect conidiophores or synnemata has been thought to be conducive to spore production and dispersal in the aquatic environment (Goh, 1997). Goh also suggested that the long conidiophores may adjust in length so spores are produced above the water surface.

Some freshwater hyphomycetes produce conidia with modified appendages, setulae, or arms. *Dictyochaeta*, *Nawawia*, and *Obeliospora* produce setulate conidia. *Sporidesmiella cornuta* produce conidia with arms. *Dactylaria tunicata* and *Delortia palmicola* produce conidia surrounded by a hyaline mucilaginous sheath (Goh and Hyde, 1997). These adaptations are found in taxa in both tropical and temperate regions.

5.3.3 Why There Are More Tropical Freshwater Ascomycetes with Special Adaptations

There appears to be a greater number of tropical freshwater ascomycetes with spore adaptations for dispersal than temperate species. One reason for these greater adaptations may be due to the fact that tropical steams are more likely to be subject to spate when submerged substrata are flushed onto the bank (Hyde and Goh, 2003). Active spore

dispersal in air may then occur (Hyde and Goh, 2003). The massive ascal ring in some tropical freshwater fungi (e.g., species of Annulatascaceae) may play an important role in the dispersal of spores that can be ejected back into the river. Tropical rivers may also have more turbulent events, creating a need for more highly developed attachment mechanisms, i.e., appendages and sheaths, in spores.

We have shown that in most cases tropical fungi have propagules with similar dispersal adaptations to those of temperate fungi. The tropical freshwater ascomycetes appear to have a greater number of special features for dispersal, and far fewer temperate freshwater fungi appear to have ascospores with sheaths or appendages. Because of the higher diversity of fungi in the tropics, there is a greater range of adaptive features in their propagules. Furthermore, heavy tropical rains and large amounts of rain splash may mean that sheaths and appendages are more important for dispersal and subsequent attachment in the tropics.

5.4 FUNCTIONAL ROLE OF TROPICAL FUNGI

The role of fungi in the tropics is similar to the role of fungi in other climates, as they are plant and animal pathogens, endophytes, and saprobes (Hyde, 1997). Fungi also form mycorrhizal associations with plants and are symbionts with algae (lichens) and animals (gut fungi). One situation unique to the tropics is the fungi that live in association with termites and leaf-cutting ants (Kendrick, 1992). The termites and ants harvest lignocellulosic material (leaves and wood) and prepare fungus gardens in their nests and mounds. The fungi degrade the substrata, and the ants and termites feed on the fungi to obtain their nutrition. Although the functional roles of most tropical fungi are likely to be identical to those of their temperate counterparts, there is evidence from the tropics that fungal diversity may be linked to fungal presence as endophytes within plants; this is discussed below.

5.5 DO FUNGI PLAY A GREATER ROLE THROUGHOUT THE VERTICAL STRATA IN THE FOREST?

Fungi may play an important role in the vertical stratification of forest functions, and this hypothesis is explored here. One very good example of fungal communities being vertically stratified is apparent in mangrove ecosystems and grasses growing in intertidal regions (Hyde, 1989, 1990; Poon and Hyde, 1998b). This is illustrated using examples from Brunei mangroves and Hong Kong intertidal grasses. However, whether a similar vertical stratification of communities can be found in forests is questionable and will be discussed using data from the literature.

5.5.1 Mangrove Fungi

Fungi in mangroves are a good example of vertical stratification in tropical forests (Hyde, 1988, 1989, 1990). The vertical stratification is, however, related to habitat rather than height from the ground. Basically, there are three groups of lignicolous fungi in mangroves: those that develop in substrata that are usually submerged in saline waters and are waterlogged; those that exist on substrata that are rarely inundated with saline water and are periodically dry; and those above the high tidal level that are not inundated at any time. The first two groups are marine fungi that have the ability to exist in seawater; although the latter group may be subjected to salt spray, most are truly terrestrial fungi developing out of saline water.

5.5.2 Intertidal Grass Fungi

The intertidal distribution of fungi on intertidal grasses *Phragmites australis* in the tropics was invesigated by Poon and Hyde (1998b). They observed patterns of vertical distribution as reported by Kohlmeyer et al. (1995a, 1995b) for *Juncus* (Juncaceae). Poon and Hyde (1998b) found 3 groups of fungi: submerged, intertidal, and aerial terrestrial. *Lignicola laevis*, a common marine fungi, was confined to the basal portion of the intertidal standing senescent stems and leaf sheaths of *Phragmites australis*. Common terrestrial fungi such as *Chaetomium globosum* and *Gliomastix* sp. were found to colonize and be restricted to the apical portion of senescent stems and leaf sheaths of *P. australis*. In the middle portion of the plant, where the water–air interface occurred, intertidal fungi were present. Gessner (1977) studied the intertidal distribution of fungi on *Spartina alterniflora* in Rhode Island, a temperate region. Terrestrial and marine fungi were also found on the apical and basal portions, respectively, of the *S. alterniflora*. The fungal communities on these tropical vs. temperate hosts, however, were distinctly different, with only one species overlapping (Poon and Hyde, 1998a, 1998b). For example, no anamorphic fungi were found commonly colonizing the basal portions of the *S. alterniflora* in temperate Rhode Island, while five aquatic anamorphs were common on the basal portions of *P. australis* in subtropical Hong Kong.

5.5.2.1 Distribution of Intertidal Fungi in the Tropics

Intertidal distribution of grass fungi in freshwater habitats was studied by Wong and Hyde (2002) on the grass *Phragmites australis* and sedge *Schoenoplectus litoralis* in Hong Kong. Their results show that a greater diversity of species exists in the aerial plant parts than in the submerged plant parts, as observed in temperate regions (Tanaka, 1991; Bärlocher and Biddiscombe, 1996). Some typical freshwater taxa, such as *Aniptodera chesapeakensis* and *Halosarpheia aquadulcis*, were found on submerged parts, while terrestrial taxa such as *Cladosporium cladosporioides*, *Dictyochaeta phragmitis*, and *Tetraploa aristata* were found on the aerial parts.

5.5.2.2 Why Are Fungi Vertically Distributed?

Environmental, physiological, and biological characteristics were thought to be factors that influenced the vertical distribution of fungi (Dix and Webster, 1995). Fungi generally require a high relative humidity for growth and sporulation (Magan and Lacey, 1984). The contrasting physical conditions, resulting from a humidity gradient along a standing culm of grass, are likely to be the most important environmental factor in determining the vertical distribution (Hyde et al., 2002; Wong and Hyde, 2002). The progressive physiological aging of the plant from the apex to the base has also been correlated with variations in nutritional content, which will affect the fungal communities (Dix and Webster, 1995). Biological factors such as fungal adaptations and interactions may also be important in determining fungal communities, as well as the pattern of vertical distribution (Shearer, 1995; Hyde and Goh, 2003).

We have illustrated vertical distribution of fungi at the waterline, but in tropical ecosystems this vertical stratification may extend up into the canopy under climatic conditions of high moisture and temperature. These conditions are more conducive to fungal growth than the desiccating conditions found high up in more temperate forest systems. As far as we are aware, no studies have investigated vertical stratification of fungi in rain forests, most likely due to the difficulties involved in sampling. A greater diversity of fungi occurs at the base than at the apex of culms off bamboo, and this is thought to

be related to humidity (Hyde et al., 2002). No correlation has also been attempted to establish if leaves from different heights in the tree canopy have different fungal communities once they fall to the ground. Promputtha et al. (personal communication), however, placed leaves of a rain forest tree on the ground under the tree as well as hanging from the lower branches of the same tree and observed no significant differences in succession of fungal communities, thus indicating that vertical stratification may not be great.

5.6 PHYSIOLOGICAL CHARACTERISTICS OF TROPICAL FUNGI

5.6.1 Competition Interactions

Previous studies have shown that competitive interactions are important in determining fungal communities in temperate regions (Shearer and Zare, 1988; Asthana and Shearer, 1990; Wicklow, 1992; Dix and Webster, 1995), and this is unlikely to differ in the tropics. Yuen et al. (1999a) examined interspecific reactions in culture using 27 species of tropical and subtropical freshwater fungi and showed that competitive interactions are important in tropical fungi. Slow-extending fungi were found to be more competitive than fast-extending fungi. Some fungi-producing pigments, such as *Pseudohalonectria longirostrum* and *Kirschsteiniothelia elasterascus*, were found to be strongly inhibitory species. Freshwater hyphomycetes, however, were found to be weakly inhibitory fungi. Yuen et al. (1999b) indicated that persistent and late colonizers are more likely to produce antagonistic substances so as to inhibit the growth of early colonizers. Fryar et al. (2001) also tested the competition of specific tropical fungi with native mycota, and the results showed that inoculated fungi on wood blocks usually inhibited the native mycota. The competitive interactions of fungi are likely to be similar in both temperate and tropical regions.

5.6.2 Wood Decomposition

Soft rot, white rot, and brown rot are three major types of wood degradation by fungi (Pointing, 1999). In terrestrial habitats, brown rot and white rot play the main role in wood decay (Harmon et al., 1986; Nilsson et al., 1989). This kind of wood degradation is well documented (Käärik, 1974; Pointing, 2001). Brown- and white-rot fungi, however, are not successful in aquatic environments; therefore, soft-rot fungi play the major role in wood decay in aquatic environments. There have been several studies on wood decay in freshwater habitats in tropical and temperate regions, and the mechanism of soft-rot decay is similar in both but is carried out by different species (Yuen et al., 1999b).

5.6.3 Growth Rates

It has been shown that temperate freshwater fungi have the maximum hyphal extension between 20 and 25°C. In the case of tropical fungi, optimal growth rates also occurred between 20 and 25°C for most taxa, although some were as low as 15°C and some as high as 30°C (Koske and Duncan, 1974; Zare and Shearer, 1988a, 1988b; Yuen et al., 1998). The optimal temperature for growth of temperate and tropical freshwater taxa is therefore similar. Yuen et al. (1998) found that tropical freshwater fungi did not grow well at temperatures lower than 20°C. This may be why fungal communities in the tropics differ from those in temperate regions, as tropical species would be unsuccessful in colder regions. At a higher temperature (25°C), the optimum temperature for growth of temperate species was similar to tropical species; however, the hyphae of tropical species extended almost twice as fast. It is therefore unlikely that temperate fungi can compete with tropical fungi in tropical environments, and this is maybe a reason for their absence in the tropics.

5.7 WHAT FACTORS ARE RESPONSIBLE FOR MAINTAINING FUNGAL COMMUNITIES?

Fungi are important contributors to primary productivity and nutrient cycling in many tropical ecosystems (Wong et al., 1998; Hyde et al., 1998a). Fungal communities and their populations may be greatly affected by a number of factors. Maintaining a particular fungal community, which does inevitably change continually in both time and space through succession, is a complex and multidimensional process often linked to a number of ecological factors (Strong, 1992; Lodge and Cantrell, 1995). From a fungal viewpoint, there are some important aspects that help establish and maintain a fungal community; these include how species arrive, colonize substrates, and exploit and compete for available resources. They then perish or remain dormant during unfavorable environmental conditions (Strong, 1992).

Access to nutrient resources is the first priority for all species in a fungal community to colonize a substratum (Dix and Webster, 1995). Fungi that can break the physical and chemical barriers to gain access to a resource use the nutrients and maintain command over available resources for a certain time period. Nitrogen, carbon, and water are the three most limiting abiotic resources in tropical region ecosystems (Lodge and Cantrell, 1995; Suberkropp, 1995; Koide and Kabir, 2001). In many cases it has been observed that spore abundance and fungal colonization in a fungal community are primarily affected by moisture availability. Species composition of a fungal community is related to the size and quantity of available resources. Once all niches are filled, the arrival and establishment of a new species or fungal community would have to be balanced by the departure of an already established one (Wildman, 1992). Another important factor is the way in which a whole fungal community uses a particular substrate in order to maintain itself for a period. To date, there is no conclusive report of community-level substrate utilization profiles in fungi.

5.8 HOST SPECIFICITY, EXCLUSIVITY, AND RECURRENCE

The overall forest architecture may be a good predictor for determining and maintaining fungal community in a given area by providing more resources and creating more habitats. As saprobic fungi colonize substrates and use the resources to help in the decay process, they encounter continually changing conditions, which influence fungal community and development (Boddy, 1992). It is generally assumed that tropical rain forests characterized by high tree diversity will support high fungal diversity. However, numerous controlling factors creating contrasting premises may account for fungal community numbers in the tropics.

For instance, although plant diversity in closed tropical forests (where airborne, water splash, or soil transmission of propagules between host species is inefficient) may be high, many plant species have a patchy occurrence and occur in low numbers. With few host individuals that are highly scattered, it may be a better strategy for fungi to be host generalists; otherwise, they would find it difficult to colonize new hosts (Cannon and Simmons, 2002; Lu et al., 2004). If this were the case, then fungal species diversity in tropical forests would be low.

On the other hand, endophytes may account for high fungal numbers. Endophytes live within plants as symptomless inhabitants (Ghimire and Hyde, 2004). Some may become pathogens (Brown et al., 1998; Photita et al., in press), others may inhabit fruits and cause postharvest disease (Wright et al., 1996), and yet others are thought to become

saprobes following plant death (Zhou and Hyde, 2001). The last has important implications for fungal diversity. Endophytes are likely to have evolved with their hosts as has occurred with plant pathogens and may be host specific (Shivas and Hyde, 1997). If endophytes become saprobes at plant death, the result is that fungal diversity is likely to be high because these endophytic saprobes should also be host specific (Zhou and Hyde, 2001).

Even if fungi are not host specific, they may colonize and produce fruiting bodies randomly on different potential host species (i.e., exhibiting host recurrence). This is determined by the intrinsic properties of the host and its diversity, which influence dispersal success. There have been few comparable studies of host specificity, host recurrence, and host exclusivity among tropical fungi (Zhou and Hyde, 2001). Ferrer and Gilbert (2003) investigated whether composition of fungal communities is influenced by host species. They found that abundant species were generalists (colonizing three hosts), while there were distinct differences in fungal communities among host trees, and postulated that host composition plays a role in structuring fungal community.

Bills and Polishook (1994) hypothesized that much of the host recurrence exhibited by saprobic microbial decomposers may be related to the physical and chemical properties of leaves rather than host specificity. Litter chemistry has recently been investigated to assess how this affects and maintains particular fungal community structure. Koide et al. (1998) found that water extracts of pine needles stimulated growth of *Suillus intermedius*, but inhibited growth of *Amanita rubescens.* Relatively little is known about how physical and chemical factors of fallen substrates, which are rich in organic compounds, can potentially control and maintain structure of fungal community composition. Furthermore, as leaf litter ages, there is alteration in quality and concentration of litter compounds that might affect species composition in a particular fungal community (Koide et al., 1998). Other less important factors are (1) rate of decay of leaf litter and its ability to retain nutrients and (2) tree density, which probably affects the microclimatic conditions in the canopy, which in turn affects species composition. Temperature, moisture, and gaseous composition are the other major relevant microclimatic factors that help maintain fungal communities (Undi et al., 1997). For instance, submerged substrates in aquatic environments must be suitable to allow fungal colonization and ultimately establishment of a fungal community (Sridhar and Bärlocher, 2000). An understanding of how these factors naturally maintain fungal community structure would provide a useful framework for examining fungal communities and how they differ with time and space, especially in succession studies.

5.9 FUTURE RESEARCH

Fungi are the major agents of litter decomposition and nutrient cycling in forest ecosystems. Despite the great importance of fungi to forest biomass decomposition, our current understanding of the patterns of community structure, diversity, host specificity, and distribution of tropical fungi is limited.

Although a greater fungal diversity has been recorded from temperate regions, this may not be an accurate reflection, as inventory of tropical fungi is fragmentary (Isaac et al., 1992; Rossman, 1997). Further studies on more substrates, distribution of fungi, systematic relationships, and host specificity/recurrence will improve our understanding of fungal diversity in the tropics. Whether some fungi are restricted to particular geographical locations is debatable. As discussed in this chapter, it can be seen that while some genera/species tend to have restricted geographical distributions and host recurrences, others are cosmopolitan and have a broad host range. Further work is required to assess

the biogeography of these fungi. Evidently, there are ecological factors that might affect distribution of tropical fungi worldwide (e.g., climatic conditions, competition, ease of dispersal, nutrient availability, temperature), but data for these are not well documented.

New techniques should be used to assess diversity of those fungi that cannot be cultured (or do not form fruiting bodies on the host) in order to allow a more precise assessment of currently known tropical fungal communities and their distribution. DNA-based techniques can provide a comprehensive measure of the diversity and composition of fungal communities because they survey both the cultured and often predominant nonculturable members of a community (Van Elsas et al., 2000; May et al., 2001). Surveys by polymerase chain reaction (PCR) amplification of naturally occurring rRNA and rDNA genes or functional genes, followed by cloning and sequencing, can be used to determine communities that are extremely diverse and that contain abundant noncultured representatives of novel fungi (Borneman and Hartin, 2000; Landeweert et al., 2003). Fungal community DNA fingerprinting techniques, such as terminal restriction fragment length polymorphism analysis (tRFLP), denaturing gradient gel electrophoresis (DGGE), oligonucleotide fingerprinting of rRNA genes, or single-stranded conformation polymorphism (SSCP), must be used more frequently in combination with traditional techniques to analyze fungal community composition (Egger, 1995; Lowell and Klein, 2001). This will enable mycologists to (1) characterize and identify broad-scale, consistent differences in the fungal communities in different locations, habitats, hosts, and niches and (2) investigate different ecological factors that may be involved in determining the composition of a fungal community.

Further studies will also provide a baseline for monitoring fungal community structure and dynamics with changes in environmental conditions. In addition, knowledge of the distribution of tropical endophytic fungi, arbuscular mycorrhizal fungi, and soil fungi, which are poorly documented in the tropics, await further studies.

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6

Lichens and Microfungi in Biological Soil Crusts: Community Structure, Physiology, and Ecological Functions

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6.1 INTRODUCTION

Biological soil crusts are soil surface communities of biota that live within or immediately on top of the uppermost millimeters of soil. They consist of cyanobacteria, algae, mosses, microfungi, and lichens. Cyanobacterial and microfungal filaments, rhizinae and rhizomorphs of lichens, and rhizinae and protonemata of bryophytes weave throughout the top few millimeters of soil, gluing loose soil particles together (Figure 6.1). The intimate association between soil particles and organisms forms a more or less coherent crust. A quantitative estimate of global biological crust cover is difficult to obtain and not yet available, but the worldwide coverage of the terrestrial surface by soil crusts is very high. In arid and semiarid areas, biological soil crusts may constitute up to or more than 70% of the living cover.

Lichens are an essential part of biological soil crusts. About one fifth (19%) of all known species of fungi are lichenized; that is, they form a stable symbiotic association with green algal or cyanobacterial photobionts that provide nutrients for the mycobiont. The vast majority of lichenized fungi belong to the Ascomycota, with 42% of all fungi in this group forming lichens (Kirk et al., 2001). About 85% of lichen-forming fungi are symbiotic with Chlorophyta (green algae, creating chlorolichens; Lange and Wagenitz,

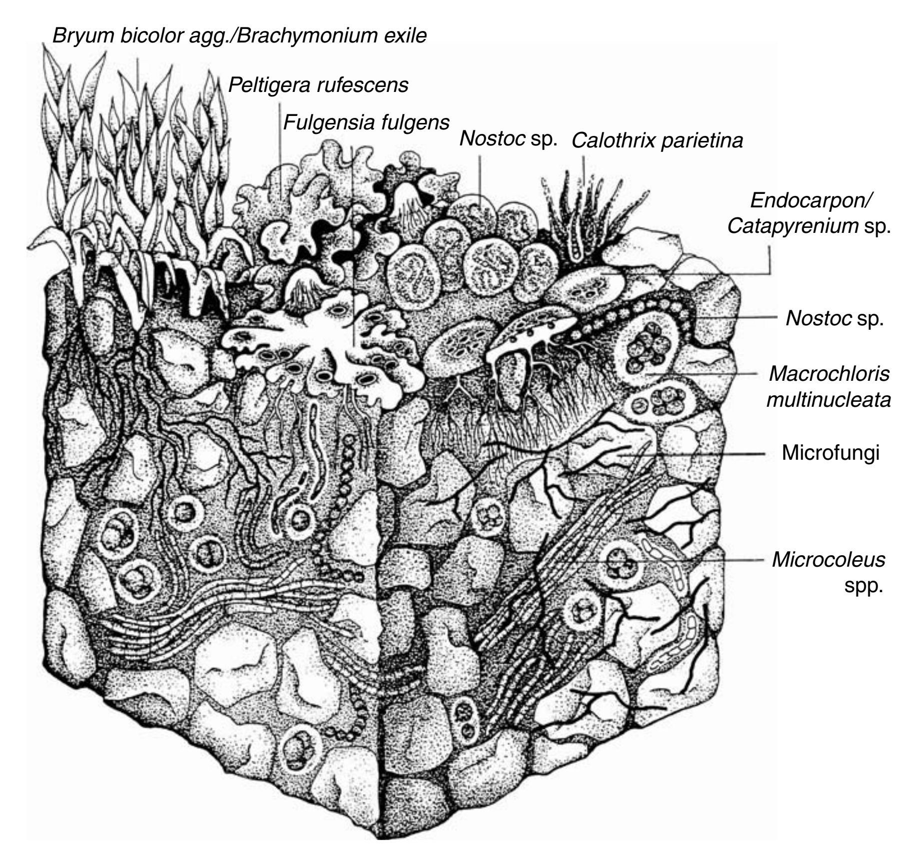


Figure 6.1 Schematic block diagram of a biological soil crust with typical colonizers. The thickness of the layer is about 3 mm, but organisms are not drawn to scale. *Peltigera rufescens* (cyanobacterial foliose lichen), *Fulgensia fulgens* (crustose–squamulose green algal lichen), and *Endocarpon* and *Catapyrenium* (placoid chlorolichens) are illustrated. (Illustration by Renate Klein-Rödder. Adapted from Belnap et al., in *Biological Soil Crusts: Structure, Function, and Management*, J. Belnap and O.L. Lange, Eds., Springer-Verlag, Berlin, 2003a, pp. 3–30.)

2003), approximately 10% with Cyanophyta (creating cyanolichens), and the remainder are associated simultaneously with both groups (Honegger, 1996). About 40 genera of photobionts have been identified in lichens: 25 are green algae and 15 are cyanobacteria.

The autotrophic lifestyle of lichenized fungi requires a long-time exposure of the green thallus to light. Most lichens are long-lived organisms with high habitat specificity. They are especially ecologically successful in polar, alpine, and warm arid and semiarid areas where competition with phanerogamous vegetation is reduced. It is estimated that approximately 8% of the Earth's terrestrial surface has lichens as its most dominant life-form (Ahmadjian, 1995). One of their most important habitats is biological soil crusts, which lichens often dominate.

In the present chapter we concentrate on those widely distributed crusts in which lichens play a dominating role. We describe their community structure, analyze the special properties of lichens as key members of biological soil crusts, discuss lichen function within the crusts, and then discuss the function of lichen-rich soil crusts as components of larger ecosystems and landscapes (for details and specific literature, see Belnap and Lange, 2003).

6.2 COMMUNITY STRUCTURE OF SOIL CRUSTS AND THEIR DISTRIBUTION

The low moisture requirement of biological soil crust organisms enables them to exist where moisture deficits limit vascular plant cover and productivity. Therefore, biological soil crusts occur in almost all ecoregions where light can reach the soil surface either temporarily (e.g., tree fall gaps) or permanently (e.g., deserts). This is true on a global, regional, landscape, and microsite scale. Thus, crust communities occur in a large variety of vegetation zones worldwide, including winter-cold steppes, grasslands, and most conspicuously, hot and cold semiarid and arid areas where plants are widely spaced. Vegetational communities in these more arid regions range from evergreen and deciduous woodlands, saltbush communities, grassland, and shrub and succulent formations to areas with fixed dunes or where vascular plants are restricted to water-collecting depressions. Soil crust communities also colonize the spaces between vascular plants in polar and alpine areas. On a small scale, soil crust communities are even found in temperate climatic regions, such as xerothermic local steppe formations in Central Europe and in the pine barrens of the U.S.

Biological soil crusts can be grouped into four types, based on habitat conditions, taxonomic composition, physical appearance, and function. *Smooth* crusts are found in hyperarid regions (e.g., hyperarid Australia, Atacama and Negev Deserts), where soils do not freeze. High potential evapotranspiration (PET) prevents growth of lichens and mosses, except in a few moist microhabitats. Thus, these crusts are almost exclusively endedaphic cyanobacteria, algae, and microfungi, and they actually smooth the soil surface. The other three crust categories generally have epedaphic colonizers such as lichens and mosses in addition to the endedaphic biota. *Rugose* crusts occur in areas with lower PET than smooth crusts. Although dominated by endedaphic cyanobacteria, algae, and microfungi, they also support scattered clumps of lichens and mosses that give the soils a slightly roughened surface (<2 cm of roughness). This crust type is found in hot deserts that lack soil freezing (e.g., Sonoran and Mojave Deserts, southern Australia, Central Negev, coastal fog zone of the Namib, and Mediterranean-type climates). *Pinnacled* and *rolling* crusts occur in regions with lower PET than rugose or smooth crusts and where soils freeze annually. Pinnacled crusts are dominated by cyanobacteria but locally can have up to 40% lichen-moss cover. Soils are frost-heaved upward and then differentially eroded downward, creating pinnacles up to 15 cm high. This crust type occurs in regions such as the Colorado Plateau and central Great Basin, U.S., and the mid-latitudes in China. Rolling crusts occur in regions where relatively low PET results in fairly continuous lichen-moss cover that is frost-heaved upward in winter. Unlike pinnacled crusts, the cohesive lichen-moss mat resists downward erosion, creating gently rolling surfaces up to 5 cm high. This type of crust is widely distributed in the northern Great Basin, U.S., and in the steppes of the Eurasian subcontinent.

Desert habitats with fog and dew (as in the Namib and Central Negev Deserts) favor chlorolichens, whereas lack of dew, less rain, and higher temperatures (as in the Arava Valley, Dead Sea area) favor cyanolichens (Galun et al., 1982). Lichens grow on almost all soil types across the pH gradient, although species composition may change. Extensive lichen cover is found on highly stable soils, such as gypsum and calcite, which also have high water-holding capacity and high levels of phosphorus and sulfur.

The floristic inventory of soil crusts of the world is still poorly known. Nevertheless, the number of crust-building lichen genera now known is already surprisingly high. Büdel (2003) reports 69 green algal lichen genera and 35 cyanobacterial lichen genera from soil crusts around the world. It is striking how similar biological soil crusts are throughout the

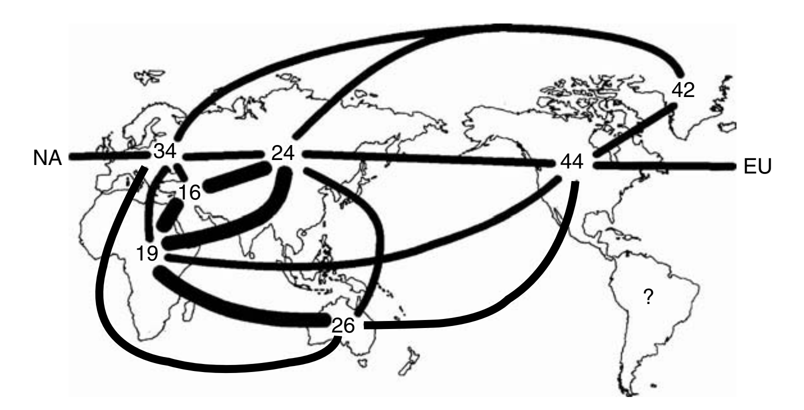


Figure 6.2 Floristic similarity on a genus basis between lichens in soil crust communities in the different areas of the world. The magnitude of the Sörensen coefficient (see text) is indicated by lines: thick lines, 60 to 90% similarity; thin lines, 40 to 60% similarity. Number of lichen species in South America is unknown. Numbers indicate the absolute number of genera. NA = North America; EU = Europe. (From Büdel, in *Biological Soil Crusts: Structure, Function, and Management*, J. Belnap and O.L. Lange, Eds., Springer-Verlag, Berlin, 2003, pp. 141–152. With permission.)

world. This is true not only with respect to the structural appearance of the communities, but also in terms of taxonomic composition. Büdel (2003) calculated the floristic similarity of soil crust lichens on a generic basis using a Sörensen coefficient (the ratio of identical genera to the sum of all lichen genera present; Figure 6.2). Soil crust lichens show several strong floristic relationships among continents, with a generic similarity of 60 to 90% between Asia, the Middle East, and Africa. A similar relationship exists between Africa and Australia. The relationship among the other continents, although weaker, is still high. There are even some lichen species (e.g., *Psora decipiens*, *Collema tenax*) with a worldwide distribution and that occur in soil crust communities on almost all continents.

Representatives of almost all types of lichen growth forms can be found in soil crust communities. Crustose lichens cover the soil with an appressed, more or less flat layer of thalli. More or less isolated, crustose thallus scales occur in placoid genera (such as *Psora*, *Buellia*, and *Trapelia*), and shield-like scales can form peltate thalli that are attached by a central holdfast (e.g., *Endocarpon*, *Peltula*). When thalli are more continuous the thallus surface is usually divided into small areoles (e.g., *Diploschistes*, *Lecidella*, *Acarospora*). Squamulose genera such as *Squamarina* represent a transition to the foliose lichens. Here, the margins of the individual thallus lobes are raised above the substrate (e.g., *Peltigera*, *Xanthoparmelia*). The transition to the fruticose form is represented by genera such as *Toninia* with inflated thallus lobes, whereas examples of soil crust fruticose species include *Cladonia* and *Cladia* species. Most of these lichens have a heteromerous (stratified) structure. Several cyanobacterial lichens have homoiomerous (unstratified) thalli and a gelatinous consistency, with the most important species of this group belonging to the genus *Collema*.

6.3 ECOPHYSIOLOGICAL FUNCTIONING OF SOIL CRUST LICHENS

The microenvironment in which the soil crust biota are found, the soil surface, is one of the most extreme habitats for autotrophic organisms on Earth. Here, the danger exists that high levels of solar radiation might damage tissue and DNA and might cause photoinhi-

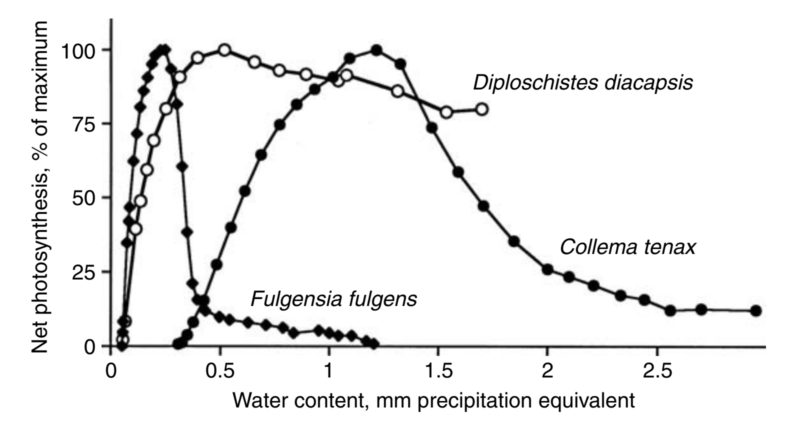


Figure 6.3 Dependence of net photosynthesis (percent of maximum) on water content for different types of soil crust lichens: *Fulgensia fulgens* (from local steppe formation, Würzburg, Germany) and *Diploschistes diacapsis* and *Collema tenax* (both from Colorado Plateau, UT). (From Lange, in *Biological Soil Crusts: Structure, Function, and Management*, J. Belnap and O.L. Lange, Eds., Springer-Verlag, Berlin, 2003, pp. 217–240. With permission.)

bition. This zone is also where the highest and lowest temperatures occur within the soil atmosphere profile. Even in temperate regions, the temperatures of lichen thalli at the same site can be up to 70°C in summer and down to –20°C in winter. Temperatures are likely to be even more extreme for soil crust lichens in hot deserts or in polar habitats. Thus, the ability to tolerate extreme temperatures (at least in the desiccated state) is a requirement for soil crust organisms. All soil crust components are poikilohydric and are often exposed to long periods of strong dehydration between infrequent moistening events. *Cladonia convoluta* from a soil crust site in southwest Germany was not impaired after 56 weeks of experimental drying (Lange, 1953). Dry-weight-related water content of lichen thalli can reach 5% or less, terminating all metabolic processes. In deserts, precipitation events are infrequent and generally less than 3 mm (Sala and Laurenroth, 1982). Therefore, lichens must be able to use these small events, as well as snowmelt, fog, dew condensation, or even high air humidity, for reactivation and photosynthesis.

6.3.1 Carbon Exchange

Water content (WC) is the most important parameter that determines photosynthetic productivity of a soil crust lichen. The moisture compensation point (MCP) denotes the minimal WC that is required to reach positive net photosynthesis (NP), while optimal WC results in maximal rates of NP. The water-holding capacity of a lichen is the maximal amount of water that can be absorbed by the lichen thallus. Various species have different thallus structures and specific physiological features that result in large differences in WC, MCP, and NP. Thus, individual species have very different carbon exchange response patterns (Figure 6.3). The chlorolichen *Fulgensia fulgens,* with a very low MCP, is capable of using very slight hydration by dew or fog (Lange et al., 1997). This species is even able to reactivate photosynthesis by using water vapor from very humid air, i.e., without moistening by liquid water. However, the water-holding capacity of *F. fulgens* is low and NP is heavily depressed at high thallus water content (suprasaturation), as the presence of water increases the diffusion resistance for CO_2 uptake. Lichen types such as *Fulgensia* are best adapted to regions where small, nonrain moisture events are frequent. In contrast, both the moisture requirement and water-holding capacity of the

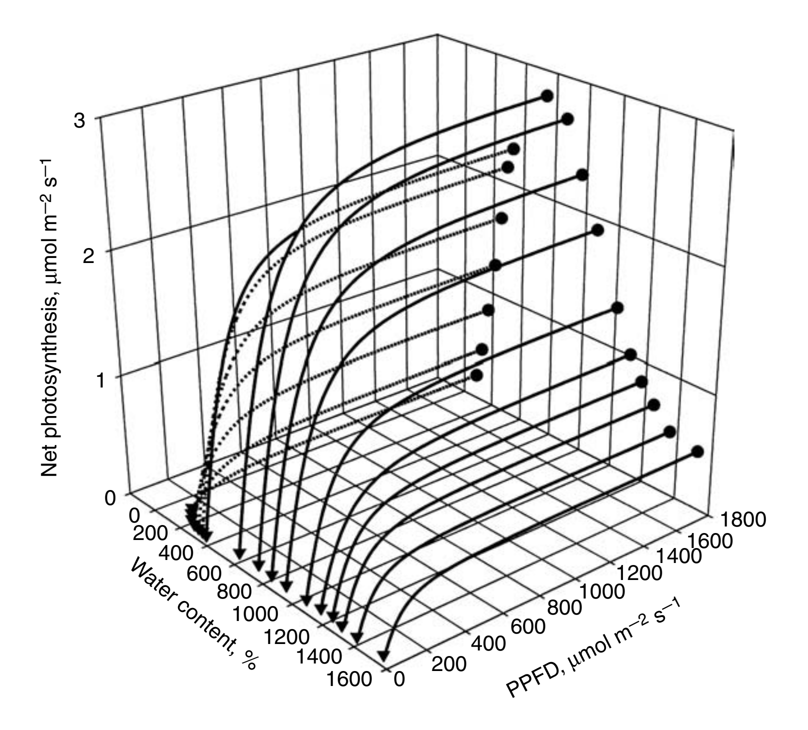


Figure 6.4 Response of net photosynthesis (at 17°C) to PPFD at several thallus water contents (percent of dry weight) for *Collema cristatum* (local steppe formation, Würzburg, Germany). (From Lange et al., *Journal of Experimental Botany*, 52, 2033–2042, 2001. With permission.)

gelatinous cyanobacterial lichen *Collema tenax* are much higher (Lange, 2000). This species begins photosynthetic carbon gain at a WC that is higher than the optimal hydration for *Fulgensia*. Obtaining such a high WC usually requires a rain or snow event. However, with its high water storage, *Collema* can make better use of these larger moisture events, giving it long-lasting periods of activity. Such cyanobacterial lichens are frequently found in deserts and semideserts where rain is the predominant source of moisture, even if it is sparse. *Diploschistes* species are highly favored in all habitats due to lack of suprasaturation depression and through substantial water-holding capacity. Soil crust species of this genus are very widely distributed, ranging from the temperate and Mediterranean regions, across different types of deserts, and to the cold steppe formations in Asia and the U.S.

Typical light response curves of soil crust lichen's NP reveal sun plant characteristics with relatively high light compensation points and light saturation points that exceed 1000 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Figure 6.4 shows a suite of light curves at different degrees of hydration for a *Collema* species. There is no observable photoinhibition even at the highest light levels. Maximal NP rates are attained at optimal WC of 600% of thallus dry weight. The character of the light curves remains identical at suboptimal WC when photosynthesis becomes increasingly limited by desiccation, as well as at supraoptimal WC when thallus diffusion resistance increases.

The different types of terrestrial lichens can tolerate a large range of temperatures for effective net photosynthetic productivity. Upper temperature compensation points for CO_2 exchange are very high (>40°C) for cyanobacterial lichens and slightly lower for chlorolichens (~30 to 35°C; see Figure 35.2 in Chapter 35). For some of the green algal

soil lichens, maintenance of low, but still measurable, rates of net CO_2 fixation could be detected under controlled conditions (Lange, 1965) down to thallus temperatures of –12°C (*Cladonia rangiformis* from central Germany) and –22°C (*Cladonia convoluta* from the Mediterranean area of southern France).

Photosynthetic carbon gain and respiratory carbon loss under field conditions are the result of a complicated interplay between environmental conditions and the functional response patterns of individual soil crust species. Certain characteristic weather conditions occur repeatedly and have resulted in four main types of diel courses of CO_2 exchange in soil crust lichens. (There are also dry days without any metabolic activity.) These four response types are illustrated with typical days for *Cladonia convoluta* from the local steppe formation of Würzburg, Germany (Figure 6.5). Panel a shows that moistening by dew, frost, or high air humidity results in a very short peak of NP in the early morning hours. In panel b, one can see that thorough wetting with rain during the night enables the lichen to be active longer (the next morning once light conditions become favorable) than when wetted with dew, until the thallus desiccates again. Panel c portrays the frequent changing of moist and dry periods due to the quick responses of the lichen's metabolism, and the most productive situation for *C. convoluta*. Panel d depicts days when the thallus is continuously moist under favorable light conditions. These four types of weather conditions potentially occur for soil crust lichens in many habitats and regions. However, the frequency at which each weather condition type occurs, the duration of crust activity, and the magnitude of activity rates will vary by region. Dew and fog can be the main sources of hydration for many soil crusts, especially in hot coastal deserts, and winter frost can be a significant hydration source in interior cool and cold deserts.

The photosynthetic capacity of soil crust lichens is remarkably high. Maximal rates of NP under optimal hydration, light, and temperature conditions are in the range of 7.0 (*Collema tenax*), 5.9 (*Diploschistes muscorum*, *Lecidella crystallina*), 5.5 (*Squamarina lentigera*), and 5.1 (*Fulgensia fulgens*) µmol CO_2 m^{-2} s^{-1}. This is close to the 10 to 20 µmol CO_2 m^{-2} s^{-1} typical maximal rates for light-saturated leaves of sun plants (Lange, 2003). However, in contrast to phanerogamous leaves, optimal water content is a rare and short event for poikilohydrous crust lichens, and they can only transiently make use of their high photosynthetic capacity. In addition, their photosynthetic productivity is limited due to the short and infrequent hydration times. Under temperate habitat conditions (central Germany), metabolic activity time ranges from 35 to 65% of the year, with *Fulgensia fulgens* having the lowest activity time. Photosynthesis occurs only 13 to 27% of the year, with *Fulgensia* again having the lowest activity time (Figure 6.6). On the Colorado Plateau of Utah, photosynthetically active times are estimated at 9 to 11% of the year for a *Collema* soil crust (Belnap, 2002). In the coastal fog zone of the Namib Desert, total metabolic activity time for soil crust lichens is estimated at 10 to 12% of the year, while this proportion is likely still smaller for arid regions with even less atmospheric moisture (Lange et al., 1991).

Projections from CO_2 exchange measurements in the field and from modeling efforts based on laboratory studies allow estimates of the order of magnitude of annual productivity of soil crust lichens (Table 6.1). The area-related C (carbon) balance (net primary productivity) is highest for the foliose–fruticose species *Cladonia convoluta.* Crustose Namib lichens profit from low respiratory carbon losses such that their production is similar to the nonfruticose temperate species. For mixed-lichen or moss-dominated soil crust communities, annual C balances are estimated at 120 to 370 kg of C ha^{-2} $year^{-1}$ (Evans and Lange, 2003). This is a substantial contribution to the C budget for the semiarid and arid ecosystems where the vascular plant productivity is low.

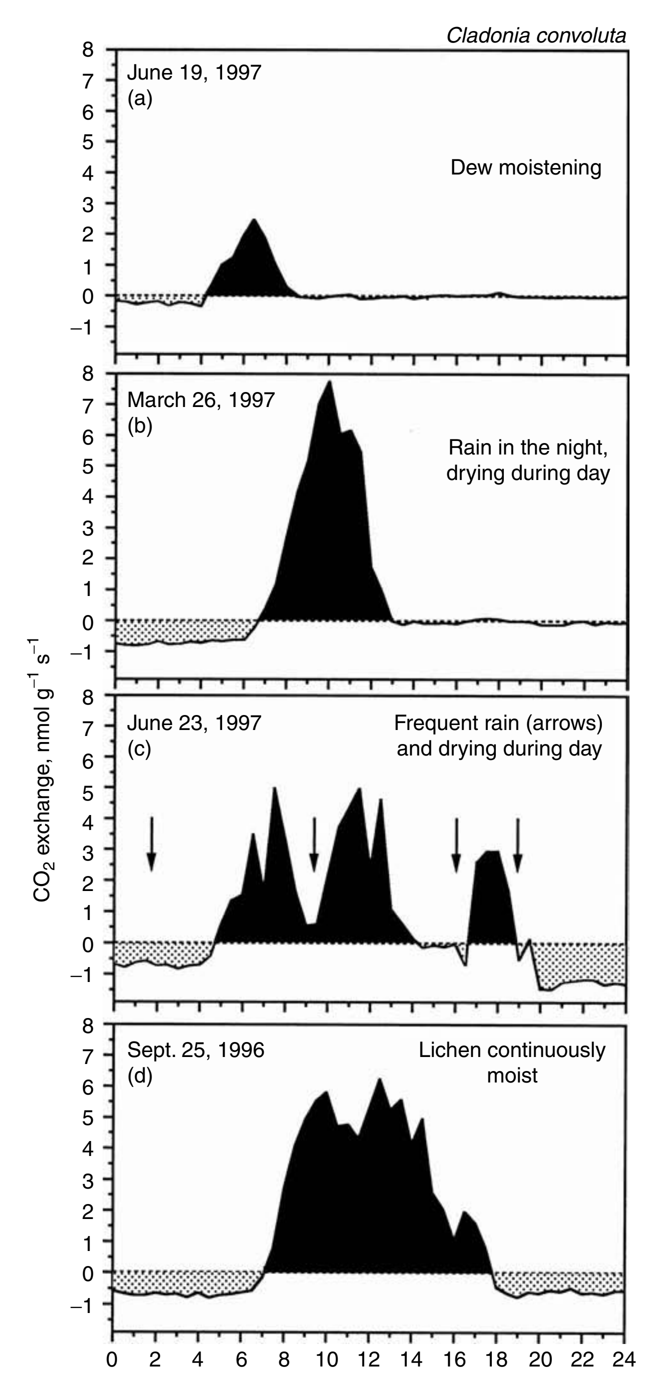


Figure 6.5 Natural diel time courses of CO_2 exchange of *Cladonia convoluta* (local steppe formation, Würzburg, Germany) that are typical for the characteristic weather types (Panels a–d, see text). (From Lange and Green, *Bibliotheca Lichenologica*, 86, 257–280, 2003. With permission.)

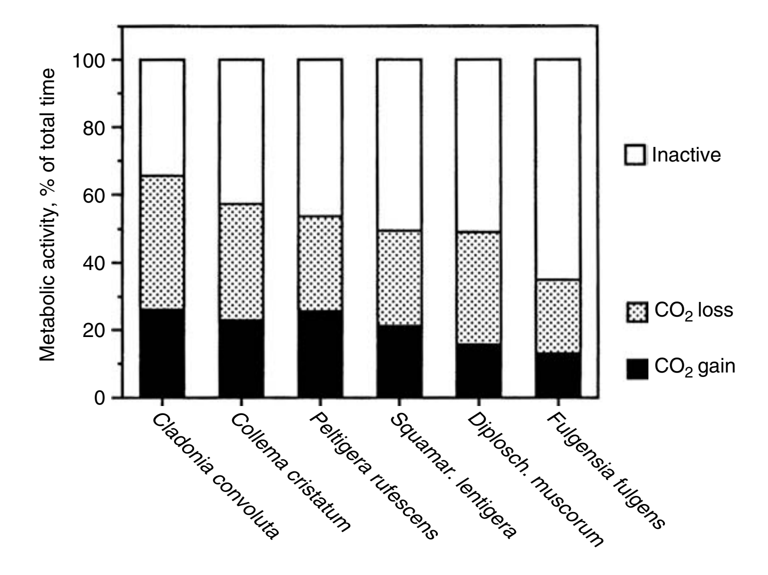


Figure 6.6 Duration of metabolic activity (metabolically active with photosynthetic CO_2 gain or respiratory CO_2 loss, respectively, or inactive) as a percentage of the total time of measurement period for different soil crust lichens (species from local steppe formation; measurements under quasi-natural conditions in the Botanical Garden at Würzburg, Germany). Data are representative for the course of 1 year. (From Lange and Green, *Bibliotheca Lichenologica*, 86, 257–280, 2003. With permission.)

Table 6.1 Estimates of Annual Carbon Budget (ΣC) for Single Lichen Thalli of *Squamarina lentigera*, *Cladonia convoluta*, *Collema cristatum* and a Community of Crustose Lichens of the Coastal Fog Zone of the Namib Desert

	ΣC[a]		
	g C m^{-2} $year^{-1}$	**mg C g_{DW}^{-1} $year^{-1}$**	**mg C $(gC)^{-1}$ $year^{-1}$**
Squamarina lentigera[b]	28.2	41.15	157.1
Cladonia convoluta[b]	142.3	98.3	225.8
Collema cristatum[b]	25.8	84.3	199.7
Crustose lichens of the Namib Desert[c]	32		
Crust communities, lower range[d]	0.4–2.3		
Crust communities, higher range[d]	12–37		

Note: The lower range of annual production estimates for cyanobacteria-dominated soil crust communities and the upper range for lichen- and moss-dominated communities are obtained from the literature (Evans and Lange, 2003).

[a] Related to projected thallus area, dry weight, and carbon content.

[b] Local steppe formation, Botanical Garden, Würzburg, Germany (Lange, 2000; Lange and Green, 2003, 2004).

[c] Lange et al., 1994.

[d] Cyanobacterial-dominated soil crust communities, lower and higher ranges of estimated annual production (Evans and Lange, 2003).

6.3.2 Nitrogen Fixation and Loss

Nitrogen (N) levels are low in desert ecosystems relative to other ecosystems. Atmospheric input is low (Peterjohn and Schlesinger, 1990), the distribution and cover of N-fixing plants is limited (Farnsworth et al., 1976), and heterotrophic bacterial fixation is also low (Wullstein, 1989). Consequently, cyanolichens and free-living soil cyanobacteria can be an important, or the dominant, source of fixed N for plants and soils in many desert ecosystems (Evans and Ehleringer, 1993). Because N can limit plant productivity in deserts (Ettershank et al., 1978; James and Jurinak, 1978; Romney et al., 1978; Nobel et al., 1988), maintaining normal N cycles is critical to maintaining the fertility of desert soils.

Most soil crusts in the western U.S. are dominated by N-fixing soil cyanobacteria (*Microcoleus*, *Scytonema*, *Nostoc*) and cyanolichens (*Collema*, *Heppia*, *Peltula*). A large range of N input has been previously reported for these organisms (reviewed in Belnap, 2003b). The most recent estimates for soil crust communities range from an average of up to 1 kg of N ha^{-1} $year^{-1}$ for *Microcoleus*-dominated cyanobacterial soil crusts to an average high of 9 kg of N ha^{-1} $year^{-1}$ for *Collema*-dominated soil crusts (Belnap, 2003b).

Nitrogen fixation is highly dependent on many factors (Figure 6.7; Belnap, 2003b). Nitrogen fixation requires the products of photosynthesis, and thus factors that influence C gain also influence N fixation. As a result, N fixation generally begins only after soil crusts have been wet, in the light, and able to fix C. Nitrogen fixation occurs mostly in the light but can also occur for a limited time (about 4 to 6 h) in the dark. Maximal N fixation rates occur at lichen water contents of approximately 20 to 80% and soil surface temperatures of about 25 to 27°C (Rychert and Skujins, 1974; Pearson et al., 1981; Paerl, 1990; Belnap et al., 1994). The timing, extent, and type of past disturbance are also a critical factor in amounts of N inputs because disturbance often reduces the biomass and flora of crusts (Belnap, 1995, 1996). Reduction of crust biomass after disturbance means fewer N inputs. Lichens (e.g., *Peltula*, *Collema*) have much higher fixation rates than a similar surface area of free-living cyanobacteria (Belnap, 2003b), but lichens are much less tolerant of disturbance than cyanobacteria. Therefore, postdisturbance crusts are generally dominated by cyanobacteria with a greatly reduced N fixation potential. Over time, as lichens recolonize and crust biomass increases, N inputs increase as well.

Nitrogen contributed by soil crusts can be lost via gas losses, overland flow, and leaching downward through the soil profile (Barger et al., in press). Recent estimates for soil crusts on the Colorado Plateau show gaseous losses to be less than 1 kg of N ha^{-1} $year^{-1}$, with losses higher under lichen crusts relative to cyanobacterial crusts. Overland flow events, which occur every few years, can remove up to 6 kg of N ha^{-1} per event for cyanobacterial crusts, mostly via large sediment losses. Losses via overland flow for lichen crusts are very low (~1 kg of N ha^{-1}), as sediment losses are limited. Nitrogen losses via leaching have not been investigated.

Nitrogen fixed by crust organisms is made available to surrounding soils and co-occuring organisms in two ways. First, N is released when the crust organisms die. Second, 5 to 88% of newly fixed N is released with wetting events into surrounding soils (Figure 6.8; reviewed in Belnap, 2003b). This N is utilized by nearby vascular plants (see next section), microbes, and invertebrates. Therefore, biological soil crusts can be an essential source of N for otherwise infertile desert soils.

6.3.3 Other Aspects of Soil Fertility

Roughened soil surfaces, protuding lichen and moss tissue, and the mucilaginous sheath material of cyanobacteria capture dust, increasing the amount of fine particles in soils (Reynolds et al., 2001). Soil crusts increase soil pH from 8 to 10.5 (Garcia-Pichel and Belnap, 1996), affecting the availability of many plant-essential nutrients. As noted above,

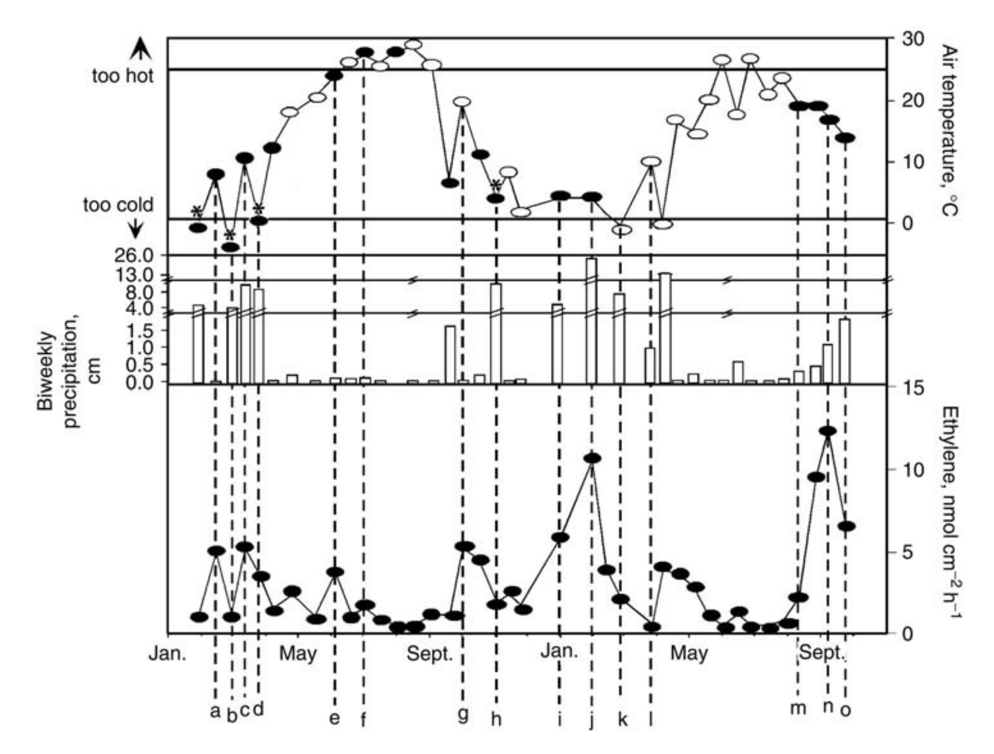


Figure 6.7 Biweekly measurement of acetylene reduction assay (ARA) for 2 consecutive years in field-collected *Collema* from southeastern Utah, measured in the lab under standard conditions (fully moistened, light, 25˚C). Top panel: Average air temperatures for the sampling date. Samples were collected in early morning. Sample dates with rainfall within 3 days of sample collection are indicated by circle color: *dark,* rain; *gray,* snow; *clear,* none; * snowmelt that added water to soils. Middle panel: Amount of precipitation. Precipitation was measured 10 miles from the sample site; thus amounts recorded estimate only those at the study site. Bottom panel: Nitrogenase activity (NA). Samples were collected, moistened, and incubated under light at 25˚C for 4 h. In general, if soils had been moist within 3 days of collection, NA levels were highly correlated with daily average temperature ($r^2 = 0.93$) unless temperatures were below 1˚C or above 26˚C. The following letters refer to the *vertical lines labeled at the bottom of the figure. (a–d),* when temperatures are above 1˚C and soils are moist, NA is observed. Even if soils are moist, low air temperatures preclude ARA activity; however, once temperatures increase, ARA activity resumes. Levels are positively correlated with temperature; (*e)* temperatures are at the maximum for NA. Soils are moist, but this moisture follows a long dry period, and thus NA levels are moderate; *(f)* air temperatures exceed the maximum, and although soils are moist, no NA is detected; (*g)* this sample point was anomalous, as no soil moisture was recorded, but moderate NA were still observed; (*h)* low temperatures and soil moisture result in low NA levels; (*i–k)* snow melts, and NA levels soar until air temperatures get too low; (*l)* in spite of optimal air temperature, lack of moisture precludes NA; *(m–o)* rain after a long dry period initiates NA. As soils continue to receive moisture, NA increases, although air temperatures are similar. As temperatures decrease, so does NA.

their presence can increase soil N by up to 200% (Shields and Durrell, 1964) and C by up to 300% (Rao and Burns, 1990; Rogers and Burns, 1994). Crusts also increase soil organic matter, known to ameliorate compaction, reduce inorganic soil crusting, reduce nutrient leaching losses, and increase soil moisture retention (Tongway and Ludwig, 1990).

Exopolymers secreted by soil crusts also modulate metal-ion concentrations at the microbial cell surface by creating a mosaic of polyfunctional metal binding sites (Greene

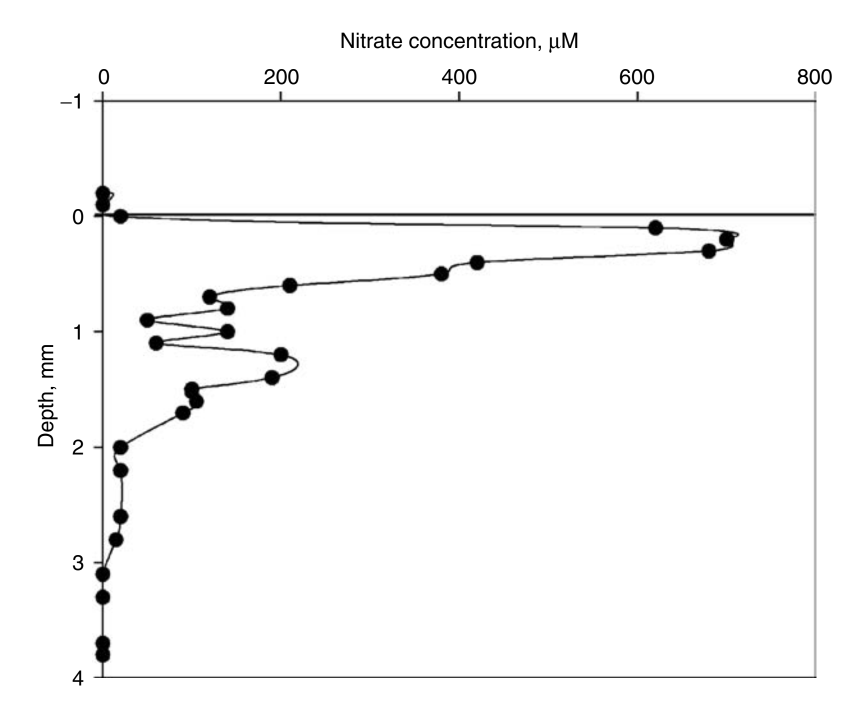


Figure 6.8 Profiles of nitrate concentrations under the lichen *Collema* within 30 min of wetting in the light. (Adapted from Garcia-Pichel and Belnap, in *Biological Soil Crusts: Structure, Function, and Management*, J. Belnap and O.L. Lange, Eds., Springer-Verlag, Berlin, 2003, pp. 193–201.)

and Darnall, 1990). These polymers act to prevent heavy metals from approaching the cell surface while concentrating growth-promoting nutrients (Lange, 1976; Geesey and Jang, 1990). Soil fines, with attached nutrients, also bind to crustal organisms. Most binding is extracellular; thus, bound nutrients remain plant-available (Geesey and Jang, 1990). Soil crust organisms secrete powerful metal chelators such as siderochromes (Schelske et al., 1962; Lange, 1974; McLean and Beveridge, 1990) that form complexes with polyvalent metals, keeping them in a plant-available form. Chelators are also effective in sequestering essential trace metals that otherwise occur at very low concentrations in the soil (Paerl, 1988). Secretion of peptide nitrogen and riboflavin combine with siderochromes to keep phosphorus, copper, zinc, and iron plant-available (Bose et al., 1971; Lange, 1974; Gadd, 1990; Geesey and Jang, 1990). Crusts also secrete glycollate (which stimulates uptake of phosphorus), various vitamins (e.g., B_{12}), auxin-like substances, and other substances that promote growth and cell division in plant and animal tissue (Fogg, 1966; Venkataraman and Neelakantan, 1967). Thus, there are many ways in which biological soil crusts increase the fertility of desert soils.

6.4 BIOLOGICAL SOIL CRUSTS AS AN ECOSYSTEM COMPONENT

6.4.1 Water Relations

Biological soil crusts influence both the infiltration and soil retention of precipitation. Infiltration is determined by a balance among the permeability of the soil surface, the water storage capacity of the soil crust organisms, and the effect of soil surface roughness

on the residence time of the water. In all deserts, the presence of crust organisms decreases soil permeability because these organisms clog soil pores. On the other hand, crust organisms increase soil permeability by forming soil aggregates that then create larger soil pores than those formed by individual soil particles. Soil crust organisms also absorb water, thereby increasing soil water storage capacity, with lichens and mosses absorbing more water than cyanobacteria. Therefore, in hot deserts where lichen–moss cover and soil surface roughness are very low, both permeability and water residence time are limited and localized infiltration is decreased. The resultant increase in runoff from crusted plant interspaces potentially provides increased water availability for nearby vascular plants, and breakup of interspace crusts can result in mortality of downslope plants (Zaady and Shachak, 1994; Eldridge et al., 2000).

In contrast, relatively undisturbed crusts in cool and cold deserts have high lichen–moss cover and high soil surface roughness. Although soil permeability is somewhat lowered by the soil crust organisms, the concomittant increase in pore size and space, soil moisture storage, and water residence time results in increased localized infiltration (Loope and Gifford, 1972; Brotherson and Rushforth, 1983; Johansen, 1986; Harper and Marble, 1988). However, it should be kept in mind that soil texture can override any effect of soil crusts. For instance, cracking clays have low infiltration rates and coarse soils have high infiltration rates, regardless of biological crust cover. The presence of soil crusts also influences the retention time of moisture once it enters the soil (George et al., 2003). Unfortunately, studies have been limited to soil crusts on coarse soils of southeast Utah. Results show that soil crusts increase soil moisture retention times compared with uncrusted soils.

6.4.2 Soil Stability

Cyanobacterial polysaccharides and the rhizines of lichens and mosses entrap and bind soil particles together. These structures can be seen firmly adhering to soil particles at up to 10 cm below the soil surface in both wet and dry soils (Figure 6.9, top; Belnap and Gardner, 1993). Soil particles are strung together or aggregated into larger particles (Figure 6.9, bottom). The heavier and larger aggregates are more difficult for wind or water to move, thus reducing erosion. In addition, these aggregated particles enable sandy soils to stay in place on steep slopes and in areas of shallow bedrock. When wetted, this biotic material swells and covers the soil surface even more extensively than when dry, protecting soils from both raindrop erosion and overland water flow.

Studies across many soil and crust types show conclusively that wind and water erosion is reduced by the presence of soil crusts, with lichen–moss crusts reducing sediment production by up to 35 times compared with cyanobacterial crusts or bare soil (Figure 6.10; Belnap and Gillette, 1997, 1998). Replacement of lost soil via newly weathered material can take 5,000 to 10,000 years in deserts (Dregne, 1983), and dust deposition is quite low in most regions (Danin and Yaalon, 1982; Reynolds et al., 2001). Therefore, keeping desert soils intact is critical to maintaining soil fertility in these regions.

Biological soil crusts likely stabilized soils in the geologic past as well. Without land plants or other factors to restrain wind speeds, newly formed soil particles would have blown away unless stabilized by organisms such as cyanobacteria. Terrestrial cyanobacteria appeared 1.1 billion years ago in the fossil record, and likely stabilized the soils then as they do today (Schwartzman and Volk, 1989). This stabilization of soils would have increased the amount of time water was held against the bedrock and, combined with the secretion of organic acids, likely accelerated bedrock weathering by up to 100 times as well (Schwartzmann and Volk, 1989). In addition, such stabilization would have facilitated the buildup of soils, thus providing habitat for the colonization of lichens, land plants and other terrestrial biota.





Figure 6.9 Scanning electron microscope images of cyanobacteria entwined with soil particles. The cyanobacteria link the soil particles together, increasing soil strength and aggregate structure. The magnification of the top photo is ×90 and of the bottom photo, ×120.

6.4.3 Interactions with Vascular Plants

The presence of biological soil crusts affects vascular plant germination and establishment differently in different locations. In hot deserts where soil crusts smooth the soil surface, seeds are easily washed and blown from plant interspaces to the nearest obstruction. This may be to the advantage of these plants, as water and nutrients also run off from these interspaces to downslope obstructions. Lab studies have shown that crusts both enhance and depress germination of seeds from the Negev Desert, depending on the species tested, although these results have not been corroborated in the field (Belnap et al., 2003b).

In cool and cold deserts, the roughened surfaces retain seeds in the plant interspace. The many small cracks in the soil crusts provide sites for small seeds to lodge, while large native seeds generally find optimal conditions by self-burial mechanisms (such as hygroscopic awns) or through being cached by rodents. Given localized infiltration, seed retention in the interspace may be to the advantage of the germinating seed. There are no studies that show crusts inhibit native seed germination in these regions. However, there is evidence that crusts inhibit the germination of large-seeded exotic plants such as *Bromus tectorum* that lack burial mechanisms. These seeds apparently require the increased moisture provided by a cover of soil or litter to germinate, which is limited when soil crusts are well

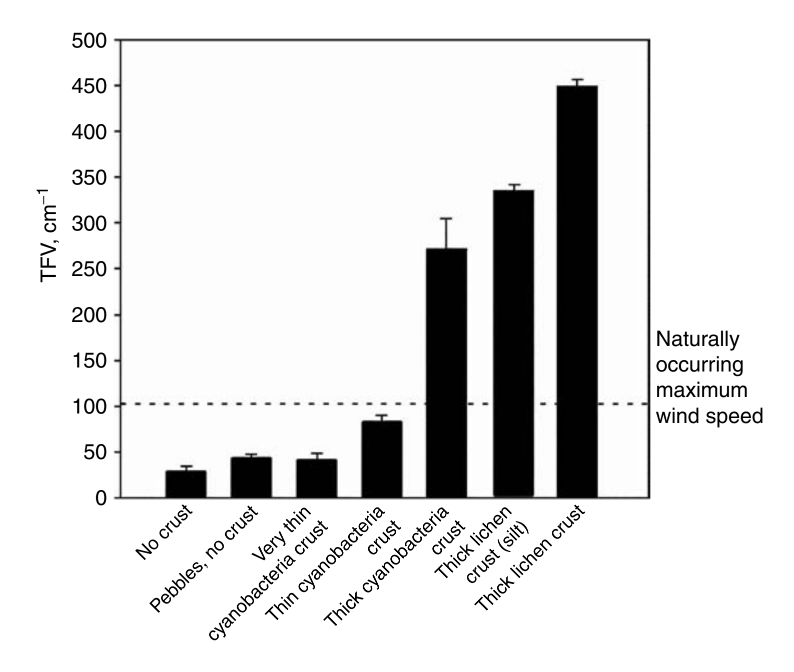


Figure 6.10 Threshold friction velocities (TFVs) of soil surfaces with different levels of biological soil crust development. (Adapted from Belnap, in *Biological Soil Crusts: Structure, Function, and Management*, J. Belnap and O.L. Lange, Eds., Springer-Verlag, Berlin, 2003a, pp. 339–347.)

developed (Larsen, 1995). However, it is important to recognize that seedling germination per se does not limit species density in desert plant communities. Rather, studies show that vascular cover in arid lands worldwide is controlled by water and nutrient availability (Tongway and Ludwig, 1990).

Biological crusts do not constitute a barrier to root penetration once seeds germinate (Belnap, unpublished data). Experiments done on both fine- and coarse-textured soils show that plant survival for forbs and grasses can be much higher, or not affected, for crusted areas than for uncrusted soils (St. Clair et al., 1984; Harper and St. Clair, 1985; Eckert et al., 1986; Harper and Marble, 1988; Lesica and Shelly, 1992). No studies have shown crusts to decrease vascular plant survival.

Plants growing on crusted soil show higher concentrations and greater total accumulation of various essential nutrients than plants growing in adjacent, uncrusted soils (reviewed in Belnap et al., 2003a). In both lab and field trials, concentrations of N and other plant-essential macronutrients in annual, biennial, and perennial plants are higher when plants grow on undisturbed crusted surfaces, compared with adjacent uncrusted sites on a variety of soil types. Dry weights of plants in both pots and the field are up to four times greater in well-developed crusts than in soils without soil crusts (Shields and Durrell, 1964; Brotherson and Rushforth, 1983; Harper and Pendleton, 1993; Belnap, 1995; Belnap and Harper, 1995).

6.4.4 Interaction with Other Soil Food Web Organisms

Cyanobacteria, lichens and mosses are the dominant primary producers in soils and also part of the soil food web. Heterotrophic bacteria and fungi are major decomposers and consumers of soil crust organisms, and their presence increases crust biomass and enhances N fixation. Grazing by protozoa also stimulates N fixation. Nitrogen provided by the

cyanolichens and cyanobacteria, in turn, increases microbial decomposition activity. Many soil food organisms use soil crust organisms as a food source. Actinomycetes, especially *Streptomyces*, protists (including amoebae, ciliates, and flagellates), nematodes, progstigmata mites, tardigrades, isopods, snails, mole crickets, tenebrionid beetles, ants, termites, isopods, snails, and mole crickets have been observed eating soil crust organisms. Fecal material from these species can also be an important source of N for the ecosystems in which they occur (Belnap, 2003b). Several studies have also shown a strong positive correlation between biological soil crusts and the mycorrhizal infection rates of vascular plants (e.g., Harper and Pendleton, 1993; Pendleton and Warren, 1996). In addition, soils underlying lichen-moss crusts have a greater abundance of organisms and more diverse soil food webs than soils underlying cyanobacterial crusts (Neher et al., unpublished).

As can be seen by the discussion above, biological soil crusts play many vital roles in the ecosystems in which they occur. They modify many aspects of soils, including roughness, fertility, hydrology, and stability, and thus influence both soil organisms and vascular plants.

6.5 ANNUAL DYNAMICS OF BIOLOGICAL SOIL CRUST LICHENS

Because most lichens are considered slow growing, many people believe that their annual dynamics are limited. Unfortunately, there has been only one monitoring study to examine this question. This study, from the Colorado Plateau, monitored soil lichen cover in 1967 and then annually from 1996 to 2002. Vascular plant cover in this habitat averages 30 to 40%, cyanobacterial cover 35 to 55%, and total lichen cover 15 to 25%. Average annual precipitation over the past 30 years has been 215 mm. Total lichen cover was similar in 1967 and 1996.

Since annual monitoring began in 1996, lichen cover has been anything but static. Figure 6.11 presents the cover of cyanolichens (*Collema tenax* and *Collema coccophorum*) and chlorolichens (*Placidium squamulosum*, *Placidium lachneum*, *Aspicilia hispida*, *Fulgensia fulgens*, and *Tonina sedifolia*). Between 1996 and 1997, the cover of all lichen groups dropped. Over the next 6 years, the cyanolichens dropped fairly consistently, for a total of 60% by 2002. Whereas the two largest drops (1997, 2002) occurred after dry years, this pattern was not entirely consistent. In contrast, the chlorolichens showed large swings in cover between 1996 and 2002. Most notably, their cover almost tripled from 2000 to 2002, despite these years occurring during the largest drought in recorded history at this site. These increases were mostly due to *Placidium* (*P. lachneum* and *P. squamulosum*), which increased 400% over the 2 years, and *A. hispida*, which increased 420% from 2001 to 2002.

The study showed a concomitant decrease in cyanolichens and increase in chlorolichens. This may be partially explained by a differential response to climate factors. There was a very high correlation between maximum June temperature and *Collema* cover ($r = 0.96$). In contrast, chlorolichens seemed to respond more to precipitation (Belnap et al., in press). There may also be competition between the two lichen groups. However, there appeared to be ample soil space for lichen colonization, although desirable habitat for these species (as yet undefined) may have been limited. Lichens are generally considered to be slow-growing organisms. While the absolute cover of lichens in the above studies was relatively small, annual changes in individual species were often very large. Therefore, this study clearly demonstrates that at least at a local level, soil crust lichen populations can be very dynamic from year to year.

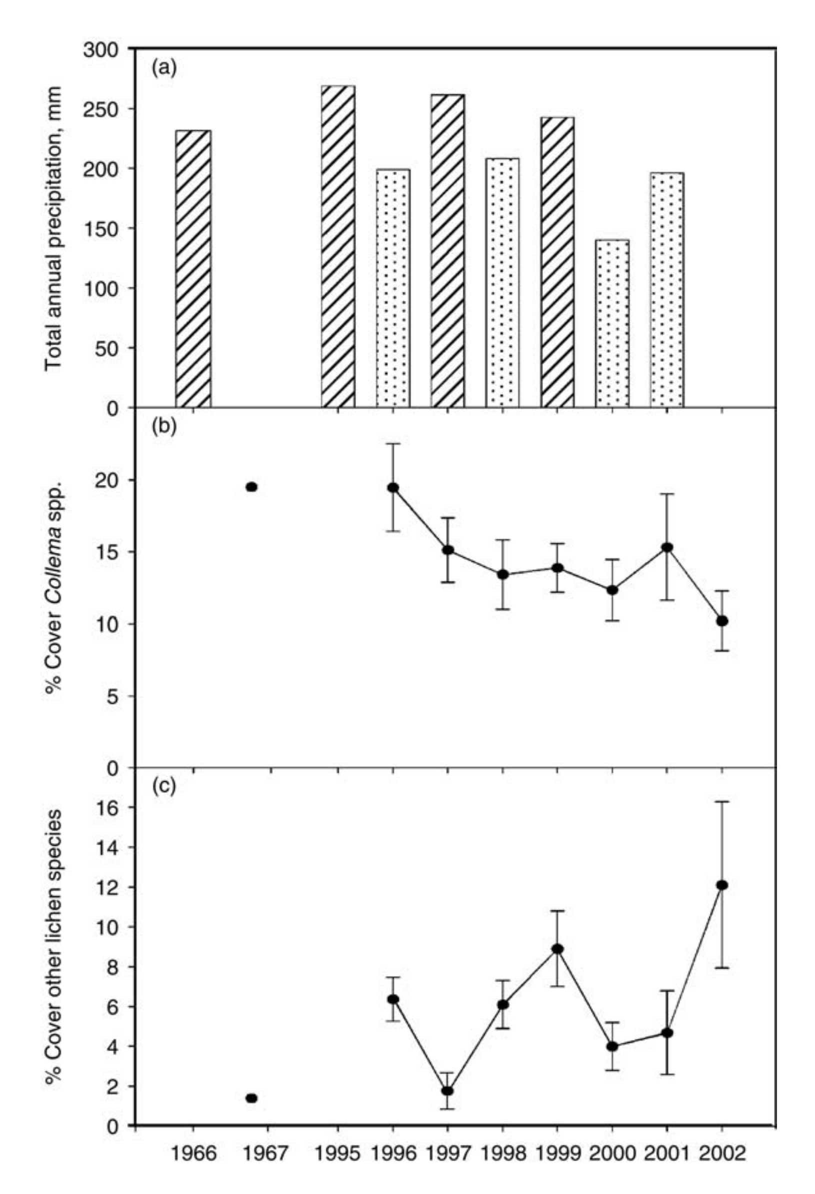


Figure 6.11 Annual dynamics of soil crust lichens from the Colorado Plateau, UT, measured in 1967 and from 1996 to 2002. Panel a: Total annual precipitation; striped bars indicate years with greater than average precipitation (>215 mm); spotted bars are years with below average precipitation (<215 mm). Panel b: Cover of *Collema tenax* and *Collema coccophorum*. Panel c: Cover of all other lichens. Note that all species declined after 1996. From 1997 to 2002, there was a large decrease in cover of the cyanolichen *Collema* and a large increase in cover of other lichen species.

6.6 CONCLUSIONS

Biological soil crusts, consisting of lichens, mosses, cyanobacteria, green algae, and microfungi, are found throughout the world. They are a critical component of the ecosystems in which they occur. The autotrophs of these soil crusts are a sink for CO_2, and the cyanobacteria or cyanobionts of lichens fix atmospheric nitrogen (N). Given that

these soil crusts cover a large portion of global terrestrial ecosystems, their metabolic activity should be considered in global carbon and nitrogen cycles. In addition, soil crusts increase the availability of other plant-essential nutrients. This effect, combined with their input of N and C to underlying soils, makes their presence especially important for plants in areas with nutrient-poor soils. Biological crusts alter soil surface properties, increasing soil stability and decreasing wind and water erosion. They affect hydrology on both a local and landscape scale. The presence of lichenized fungi and microfungi magnifies the ecological roles these soil crusts play at a given site, especially concerning the enhancement of soil fertility and stability. The effects of soil crusts reverberate throughout the ecosystems, influencing soil food webs, nutrient cycling rates, vascular plants, and faunal components. The importance of these biological soil crusts makes their monitoring and protection an important issue for landscape management, especially in arid regions.

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7

Mycorrhizal Fungi in Successional Environments: A Community Assembly Model Incorporating Host Plant, Environmental, and Biotic Filters

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7.1 INTRODUCTION

Succession in plant communities, and associated mycorrhizal fungi, can be defined as a "directional change in the composition, relative abundance, and spatial pattern of species comprising communities" (Frankland, 1992, 1998). In other words, component species replace one another as the dynamic communities change in space and time, and each species is adapted to the occupation of particular niches within the successional seres. A number of model systems have sought to follow the succession of mycorrhizal fungi. We will briefly review examples of those for background and, in turn, introduce our proposed conceptual model.

Observations of the plant colonization in successional seral environments have indicated that nonmycorrhizal or facultatively mycorrhizal plants are often first to establish in severely disturbed sites (Allen et al., 1987; Allen, 1988, 1991). There appears to be a continuum of mycorrhizal dependency along successional gradients. Nonmycorrhizal and facultatively mycorrhizal plants tend to occur and dominate in highly disturbed ecosystems. In turn, these are replaced by obligately arbuscular mycorrhizal (AM) plants, followed by ectomycorrhizal (EM) plants and ultimately ericoid mycorrhizal plant species (Read, 1989,

1992). Plant establishment thus follows a predictable pattern toward communities with a greater dependence on mycorrhizal fungi with different characteristics in their resource use, especially nitrogen (N) and phosphorus (P).

Using coastal sand dunes as a model system, Read (1989) related plant and mycorrhizal community succession to changes in soil conditions. The proportion of obligately mycorrhizal plants was found to increase with decreasing soil base status and pH. However, the periodically disturbed and nutritionally enriched high-tide line was colonized by ruderal species with minimal mycorrhizal associations. In turn, the plant communities were defined by distinctive nutrient limitations and their dominant mycorrhizal types. Plant species that were facultatively dependent on AM mycorrhizal colonization tended to occur in dune areas with limited P availability (see also Smith and Read, 1997). The extramatrical mycelium of AM fungi also stabilized the dune systems by aggregating sand and soil particles (Miller and Jastrow, 1992). The availability of AM inoculum also determines the plant community dynamics by changing the competitive balance among the early nonmycorrhizal and facultatively mycorrhizal plant species (Allen and Allen, 1984, 1988, 1990). Over time, the accumulation of soil organic matter reduces pH and inhibits nitrification. As ammonium becomes the major source of N, N replaces P as a main growth-limiting element. As a result, EM plants tend to predominate, organic matter accumulates, and base depletion proceeds. In this environment, plants with ericoid mycorrhizal associations become more important because of their ability to use nutrients bound in acidic organic complexes (Read, 1996). Read's (1989) model system elegantly relates the shifts in mycorrhizal community and plant mycorrhizal dependency to the modification of ecosystem properties during succession. However, this model does not provide a mechanistic basis to explain why certain species of mycorrhizal fungi are selected at various stages of plant community succession.

The early- and late-stage model attempts to explain the successional occurrence of EM fungi by correlation with stand or tree age (Mason et al., 1983; Dighton et al., 1986). Deacon and Fleming (1992) thoroughly reviewed this successional concept, and we will only briefly introduce it here. The early-stage fungi approximate ruderal strategies (r-selected sensu; Grime, 1979), whereas the late-stage fungi appear more stress tolerant or combative (S-selected or C-selected; Deacon and Fleming, 1992). Early-stage fungi readily colonize available host roots when their spores or mycelia are added (Fox, 1983; Mason et al., 1983) and are likely to be among the pioneering colonizers of young plants in deforested environments. As the host tree ages, early-stage fungi nearer the tree trunk are replaced by late-stage fungi. Late-stage fungi often fail to establish mycorrhizae by spores or mycelial inoculum (Deacon et al., 1983; Fox, 1983). However, they are able to dominate the root systems once established on a large tree. Furthermore, the late-stage fungi readily colonize seedlings planted adjacent to these larger trees (Fleming, 1983) but not when inoculated onto seedlings in the absence of such parent trees (Mason et al., 1983; Fleming, 1985). In this fashion, establishment only occurs from an existing food (carbon) base (Fleming, 1983; Fleming et al., 1984).

Apart from tree age, fungal succession has been attributed to differences in photosynthate availability in proximal and distal parts of the root systems (Gibson and Deacon, 1988, 1990) or the size of the food (carbon) base (Deacon and Fleming, 1992). Bruns (1995) uses a "leaky hose" analogy to explain: short roots closest to the stem receive the greatest amount of photosynthates, and the more distal roots receive only what is left after the preceding leaks. Accordingly, late-stage fungi are those requiring more host photosynthate, whereas early-stage fungi colonize roots when photosynthate availability is limited (Gibson and Deacon, 1988). Pure culture studies have confirmed that the late-stage fungi, indeed, require more sugars to grow (Gibson and Deacon, 1990). Although this model is

an elegant effort to integrate physiology of the hosts as well as their mycorrhizal fungi, it has been criticized for not acknowledging the stand-level environmental parameters that change with the age of the stand (Keizer and Arnolds, 1994; Jumpponen et al., 1999a). Furthermore, the early- and late-stage model does not incorporate the complex competitive and facilitative interactions among the soil-inhabiting microorganisms.

The concepts of fungal adaptation and the resulting environmental tolerances have often been ignored in contemporary models of fungal succession that seek to explain the occurrence of fungi based on their physiology. Deacon and Fleming (1992) also emphasized the need to resolve the more fundamental issues of fungal occurrence: To what degree is the behavior of mycorrhizae determined by soil and environmental factors? We emphasize that a comprehensive model for succession of mycorrhizal fungi must account for various aspects of fungal life strategies and their environmental tolerances. Earlier models such as r- and K-selection models focus on the reproductive output that facilitated rapid fungal invasion and establishment, the ability of fungi to tolerate stress and intensifying competition as ecosystem properties stabilized, or the increased niche overlap among component species. Instead, we aim to focus on mechanisms that explain how fungi can successfully establish and proliferate in the successional environments. Our goal is to propose a successional model that is applicable on an ecosystem scale by integrating fungal propagule availability and dispersal, host preferences and physiology, fungal environmental tolerances, and biotic interactions among mycorrhizal fungi and soil-inhabiting microorganisms (Figure 7.1). We acknowledge that such a model is a simplification of the natural successional phenomena, as we focus only on arrival of propagules and selection of mycorrhizal fungi through host, environmental, and biotic filters. Clearly, plant community dynamics and competitive and facilitative interactions, even those that are not mediated by mycorrhizal fungi, are important (Connell and Slatyer, 1977; Connell et al., 1987; Pickett et al., 1987). For example, the interactions among establishing plants and the resultant distribution of resources, including photosynthates, are likely to alter environmental conditions that influence the fungal community composition.

We also seek a mycocentric view and aim to identify the factors or processes that select the fungi that colonize hosts in successional environments. Our ultimate goal is to develop a predictive model for extant communities when data on the species pool and prevailing environmental conditions are available. We have adopted and modified concepts of assembly rules used in plant community ecology as a general ecological framework to identify processes of community assembly (Diamond, 1975; Cole, 1983; Hunt, 1991). These rules outline the constraints on the selection of community assemblages from larger local or regional species pools (Weiher and Keddy, 2001) and mechanisms and ecological processes that function to produce organismal communities (Drake et al., 1993). In other words, we seek the factors that control the community composition reflecting “both the applicant pool and the community’s admission policies” (Roughgarden, 1989, p. 218). Thus, we follow Keddy (1992) and apply these rules to emphasize the different environmental tolerances among the component fungal species.

The environmental controls are expressed as filters in our model following examples presented elsewhere (see Keddy, 1992; Weiher and Keddy, 1995, 2001). We define a filter as the biotic and abiotic environments, or their combined characteristics, that remove species otherwise available in the local and regional species pools, but lacking the ability to persist in the community under prevailing conditions (see also Grubb, 1977; van der Valk, 1981; Southwood, 1988). The use of the filter concept is particularly useful in our approach to successional ecology of mycorrhizal fungi, as it serves our overall goal to discuss the determinants of fungal persistence in successional ecosystems. To incorporate fungal dispersal and a dormant propagule bank, as proposed in Jumpponen (2003), we

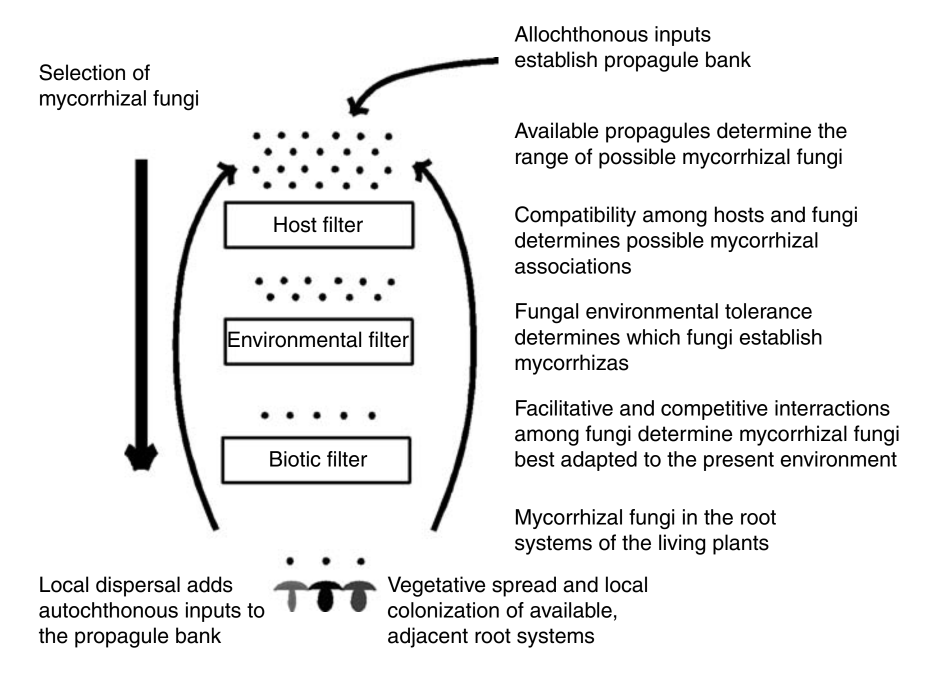


Figure 7.1 Conceptual community assembly model for mycorrhizal fungal communities during succession. Initially, out-of-site, allochthonous propagules establish available species pool (propagule bank). Successful component species are selected by filtering out those species that are incompatible with available hosts in their present physiological state (host filter), those species whose environmental tolerances do not include the prevailing conditions in the successional environment (environmental filter), and those species that are outcompeted by others in the prevailing environment (biotic filter). Species with adequate fitness to reproduce contribute to the autochthonous propagule bank via production of vegetative mycelium or via production of sexual and asexual propagules.

consider the disturbed patch (successional environment) as an island that is surrounded by a nondisturbed mainland. Severity of the disturbance within the patch determines whether any resident organic legacies (e.g., propagules, surviving individuals, organic matter, nitrogen) remain within the island after disturbance. The size of an individual island determines the scale on which dispersal mechanisms occur, namely, autochthonous vegetative spread as mycelium or within patch spore dispersal vs. an exclusive reliance on aerial or vector-mediated propagules that originate outside of the patch. Our model (Figure 7.1) concentrates on primary successional ecosystems and briefly addresses its possible relevance in secondary successional systems. Further, we limit the scope of this proposed model to EM and AM fungi, as very little is known about the successional community dynamics of other mycorrhizal fungi. The sections below are arranged to address various components of the model individually.

7.2 DISPERSAL OF FUNGI AND AVAILABILITY OF PROPAGULES IN SUCCESSIONAL ENVIRONMENTS

We will first consider primary successional ecosystems that rely primarily on allochthonous sources for species establishment (Matthews, 1992). Examples of primary successional

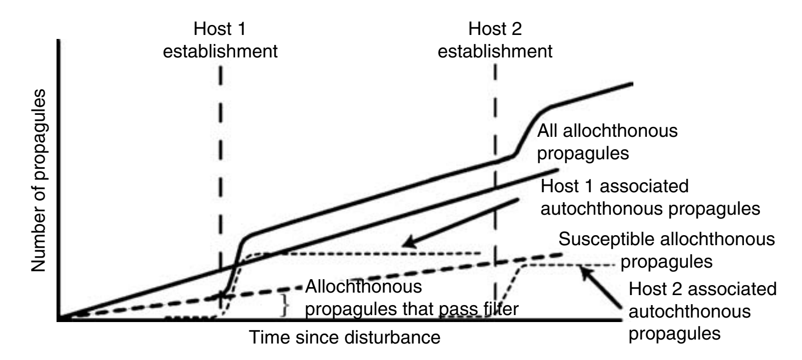


Figure 7.2 Schematic of the accumulation of mycorrhizal propagules in the propagule bank. Propagules accumulate linearly from allochthonous sources. Autochthonous propagules are produced only after functional mycorrhizal symbioses have been established. Note the increasing relative importance of autochthonous propagules over time.

ecosystems include glacier forelands, mine tailings, and volcanic substrates and islands. We will relate fungal community dynamics in these ecosystems to our successional model, and consider the relevance of this conceptual model for secondary successional ecosystems. There is little doubt that mycorrhizae can have tremendous impacts on plant growth and performance in these environments. Early transplant experiments clearly demonstrated the pivotal importance of mycorrhizal colonization: without mycorrhizae, plants often did not survive or grew at extremely slow rates (see Hatch, 1936; Trappe and Strand, 1969; Mikola, 1970). Thus, primary successional ecosystems present a challenging environment for establishment and growth of mycotrophic plants, and the availability of mycorrhizal propagules will be critical for plant succession.

Successional ecosystems vary in their availability of mycorrhizal propagules. Jumpponen et al. (2002) concluded that EM propagules on the forefront of a receding glacier were few, but their availability increased with time since deglaciation. Similarly, AM propagule numbers increased with time in pioneering communities in maritime sand dune (Nicolson and Johnston, 1979) and in mine tailing (Zak and Parkinson, 1983) ecosystems. The limited supply of infective fungal propagules in these environments underlines the importance of allochthonous sources of propagules. In large landscape fragments, such as volcanic islands or the forefronts of receding glaciers, mycorrhizal colonization propagules may be solely provided by allochthonous sources until susceptible host plants have established and thus allow autochthonous propagation (Figure 7.2).

Mycorrhizal fungi colonize the roots of as many host plants as possible and transfer (Chilvers and Gust, 1982) by vegetative dispersal (Finlay and Read, 1986a, 1986b). However, plant individuals are sparsely dispersed in primary successional ecosystems and fungal vegetative expansion between new individuals is unlikely. Therefore, in early primary successional ecosystems, the vast majority of the propagules are likely to arrive aerially and establish a dormant spore bank as hypothesized by Jumpponen (2003). Because propagule availability restricts the establishment and growth of mycorrhizal plants (Janos, 1980), autochthonous propagule production may be reduced. Stochastic events such as landslides or fecal deposits by animals may create patches of increased propagule availability (Cázares, 1992; Jumpponen et al., 2002; Kjøller and Bruns, 2003). The stochastic landscape can also determine the distribution of fungal propagules and heterogeneity: microscale topographic variation, soil surface structure, or the proximity of rocks. Both plant and mycorrhizal establishment have been shown to vary among such microsites

(Titus and del Moral, 1998; Jumpponen et al., 1999b; Titus and Tsuyuzaki, 2002). These microsites can function as safe sites for both plant and mycorrhizal propagules because infective propagules can be cached or protected, or mycorrhizal colonization of the host plants can be facilitated (Titus and Tsuyuzaki, 2002). The co-occurrence of fungal propagules and susceptible hosts may be particularly important for the successful establishment of obligately mycotrophic plants in primary successional environments (Trappe and Luoma, 1992).

Not all propagules in the propagule banks, however, are equally likely to colonize the roots of susceptible hosts. Some (early-stage) fungi can readily colonize roots of susceptible hosts when their spores are introduced into soil, whereas others (late-stage) have great difficulty establishing by spores (Deacon et al., 1983; Fox, 1983; Mason et al., 1983). The ability to colonize host roots may be controlled by host physiology (Gibson and Deacon, 1990) as well as the carbohydrate or environmental requirements of an individual fungus. In addition, some fungi are unable to form mycorrhizas as monokaryons and require dikaryotization (an anastomosis event) prior to successful mycorrhiza formation. For example, *Laccaria bicolor* (Kropp et al., 1987) and *Hebeloma cylidrosporum* (Debaud et al., 1988) were able to colonize host roots as monokaryons, whereas *Tuber melanosporum* (Rougenol and Payre, 1974) and *Suillus granulatus* (Ducamp et al., 1986) were not. Consequently, both host physiology and fungal life history govern the fungal taxa that are able to colonize the susceptible host roots in primary successional ecosystems, be it from aerial inocula or deposited by animals.

Additional factors may play important roles in the ability of propagules in the soil propagule bank to germinate and colonize susceptible roots. Soil fungistasis may inhibit propagule germination and hyphal extension in soil (Lockwood, 1977, 1992). Sensitivity to fungistasis among the fungal taxa may vary substantially (Lockwood, 1977; de Boer et al., 1998), so that not all fungal propagules will have an equal chance to establish colonization on the available host roots. We will return to fungistasis later in the section on biotic interactions. A variety of factors — including host physiology, fungal life history strategies, and soil fungistasis — can also select fungi from the soil propagule bank that are able to colonize susceptible hosts. We stress that an essential component of our model is that a variety of fungi may be present in the propagule bank, but only a limited selection of those will successfully colonize available hosts.

Propagule availability in secondary successional systems differs dramatically from that in primary successional ecosystems. Secondary successional processes may also take place in a wider variety of scales, ranging from a single windthrow to vast wildfires that (temporarily) eliminate all live vegetation over hundreds or thousands of hectares. There are various possibilities for mycorrhizal establishment after such disturbance events. Mycorrhizal colonization can establish from active mycelia that survive the disturbance event, resistant propagules other than mycelium (dormant structures, including spores and sclerotia), or similarly to primary successional systems, aerially dispersed propagules from adjacent undisturbed areas (Bruns et al., 2002). Surprisingly, Taylor and Bruns (1999) observed minimal overlap between EM community structures in mature *Pinus muricata* forest and resistant propagule banks in air-dried soil samples from the same site. Such observations suggest that any disturbance of the mycelial network may inhibit colonization by the active mycelia in secondary successional stands and result in the patchy distribution of a great diversity of fungi with differing life history strategies (Taylor and Bruns, 1999). Nonetheless, whether the mycorrhizae establish from active mycelium or a resistant propagule bank in soil, we argue that the allochthonous inoculum sources are of lesser importance than autochthonous inoculum sources in secondary successional ecosystems (see Figure 7.2). The relative importance of inoculum sources obviously depends on the

severity of the disturbance. Fast canopy fires allow the survival of surface and litter-bound mycelium, whereas the hotter surface fires typically eliminate the active mycelium that is close to the soil surface, and mycelia tends to survive only at greater soil depths (Baar et al., 1999).

Fungal life history strategies, including life span and turnover, are also likely to play an important role. Recent evidence suggests that some fungi may require frequent recolonization from newly dispersed propagules (Redecker et al., 2001). Guidot and coworkers (2002) found that *Hebeloma cylindrosporum* genets rarely, if ever, could be detected in the same locations in two consecutive samplings in coastal *Pinus pinaster* stands. These findings suggest that some fungi may establish as annual mycelia and rely nearly exclusively on reestablishment annually, whether or not the site is disturbed.

7.3 HOST FILTER

Both aspects of host physiology and susceptibility to mycorrhizal colonization vary among host species and hosts of different ages. Following Molina and coworkers (1992), we will focus on host receptivity and host range of the mycorrhizal fungi here. Clearly, these two factors will limit fungal colonization from the limited propagule banks in primary successional systems. We acknowledge that these factors are also likely to be controlled by various environmental factors that impact host physiology and performance, as well as molecular interactions between the fungus and host plant. Furthermore, the plant community structure will also influence the identity of fungal taxa residing in the propagule bank (Figure 7.2) and those fungi that establish and sustain colonization in a root system of a host (Vandenkoornhuyse et al., 2003).

7.3.1 Host Ranges of Mycorrhizal Fungi

There are very few examples of hosts that form mycorrhizae with only one species of fungi. Some dipterocarps may be an exception to this rule (Smits, 1983). Another possible exception may be members of Monotropoideae, as these plants appear to form associations with a single fungal genus or closely related group of fungi (Bidartondo and Bruns, 2001, 2002). The AM fungi were, until recently, thought to form functional associations with a wide variety of potential host species (Smith and Read, 1997), including species that are not normally considered AM hosts (see Lodge and Wentworth, 1990; Cázares and Trappe, 1993; Moyersoen and Fitter, 1999; Chen et al., 2000). However, AM fungi have been shown to be diverse and select different primary hosts even when the plants grow in mixed communities (Bever et al., 1996; Eom et al., 2000; Vandenkoornhuyse et al., 2001; Helgason et al., 2002; Husband et al., 2002; Lovelock et al., 2003). Further, different host–fungus combinations may yield symbiotic associations that are less compatible when measured in terms of benefits to each of the symbiotic partners (Molina et al., 1992; van der Heijden et al., 1998; van der Heijden and Kuyper, 2001; Bever, 2002).

Duddridge (1986) used EM host specificity as a measure of selectivity. We contend that selectivity may be most appropriate for the purposes of this contribution. Selectivity indicates the combination of processes that determine whether a fungal–host combination will yield functioning mycorrhizae (Molina et al., 1992). Different host species may select different fungi from the same soils. Newton (1991) used seedlings of *Betula* and *Quercus* to bait EM fungi from a variety of soil samples and found that different fungi colonized the seedlings, even when seedlings were planted in the same soil. Interestingly, the EM fungi also differed when hosts were planted in mixtures or monocultures (Newton, 1991). Thus, different fungi are able to establish mycorrhizae from different sources of inoculum:

a fungus that may be unable to colonize one host from propagules other than active mycelia may be able to do so when a more susceptible host is present and provides a supply of photosynthates. Similar resource limitations have also been applied to host colonization by AM fungi (Bever, 2002).

Plants within the same genus or family may be capable of hosting similar suites of EM fungi (Malajczuk et al., 1982; Molina et al., 1992). During primary succession and when propagules arrive mainly from allochthonous sources, broad-host-range fungi may be most successful. There are two main arguments why EM generalists should have a higher abundance than specialists. First, a generalist may colonize many plants and, therefore, be able to occupy a wider geographic area. Thus, the total resources potentially available for uptake and transfer to the plant are greater. Second, fungal generalists can promote the geographical expansion of a plant species because the fungal taxa tend to tolerate a broad range of environmental conditions. In secondary successional ecosystems, the case may be the opposite. Host-specific fungi may provide plants with access to exclusive pools of nutrients. For example, in the case of a stand-replacing wildfire, forests of *Pinus muricata* or *Pinus contorta* are often replaced by conspecific seedlings whose establishment depends on fire. In these ecosystems, fungi with narrower host ranges may benefit from being able to establish from the roots of fire-damaged mature trees. For instance, there may be lesser competitive potential of the nonconspecific hosts or competition from fungal taxa with broad host ranges.

Field evidence for mycorrhizal host preferences is limited. In mixed stands of EM, *Pseudotsuga menziesii–Pinus muricata* and *Pinus contorta–Picea engelmannii* illustrate that nonspecific fungi account for more than 80% of the mycorrhizal biomass in both plant taxa (Horton and Bruns, 1998; Cullings et al., 2000). Many of the same fungi also occurred on roots of the arbutoid mycorrhizal plant, *Arctostaphylos glandulosa* (Horton et al., 1999). In contrast, fungi with narrow host ranges, e.g., species of *Suillus* or *Fuscoboletinus* that are thought to be exclusively associated with *Larix* species, are unlikely to colonize a nonpreferred host under the harsh environmental conditions of severely disturbed ecosystems.

7.3.2 Host Receptivity

The receptivity of the host for mycorrhizal colonization varies greatly among host species and individuals and might be related to host age (Tonkin et al., 1989). It is also possible that receptivity is related to the early- and late-stage model discussed above. If so, receptivity would correlate with differences in photosynthate availability among mycorrhizal hosts of different ages or between proximal and distal parts of the root system (Gibson and Deacon, 1988, 1990; Deacon and Fleming, 1992). Early-stage fungi are likely to colonize younger trees or younger regions of the root systems (Gibson and Deacon, 1988, 1990), and therefore be pioneering colonizers of young plants in deforested environments. In contrast, late-stage fungi are unable to establish mycorrhizae by spores or mycelial inoculum (Deacon et al., 1983; Fox, 1983) and instead depend on an existing carbohydrate reservoir for successful establishment and colonization (Fleming, 1983; Fleming et al., 1984; Gibson and Deacon, 1988).

In this manner, the species distribution of hosts and coinciding mycorrhizal inoculum may govern the range of possible compatible host mycorrhizal fungus combinations. In a study of the responses of six early and late successional tree species to early or late successional AM inocula, all tree species had the greatest growth response to early seral fungi. However, the response to late seral inoculum varied: two tree species (*Ceiba pentandra*, *Guazuma ulmifolia*) were smallest with late seral inoculum, even smaller than the uninoculated plants, whereas the other species (*Brosimum alicastrum*, *Havardia albi-*

cans, *Acacia pennatula*, *Leucaena leucocephala*) had intermediate growth with late seral inoculum. Of these, *Brosimum*, *Havardia*, and *Ceiba* occur in late successional forest, and the others are early seral (Allen et al., in press). The host trees, through preference for fungal symbionts or changes in physiology and carbohydrate availability, selected the mycorrhizal fungi that are able to colonize and establish in their root systems. The inocula and their sources present a further challenge. In primary successional ecosystems where no live mycelial inocula possibly exist, the fungal abilities to colonize via spores and airborne propagules become critical.

7.4 ENVIRONMENTAL FILTERS

Niche can be defined as the range of physical and biological conditions, including limiting resources, necessary for a species to maintain a stable or increasing population (Hutchingson, 1957). This definition can be visualized as a multidimensional space in which each of the dimensions corresponds to an independent physical or biological variable that affects the abundance of a target species (Morin, 1999). We emphasize that environmental tolerances and niches are not static in time or space but are influenced by competitive and facilitative interactions among organisms, and interactions among different resource axes. For example, temperature has a substantial impact on the use of various carbon substrates by food and grain spoilage fungi (Lee and Magan, 1999). Here, we consider aspects of niche to include environmental tolerance (this section) as well as available resources for which mycorrhizal fungi may compete (see Section 7.5, specifically Section 7.5.3). Our environmental filters are based on the concepts of both realized and fundamental niches (Figure 7.3 to Figure 7.5).

7.4.1 Environmental Tolerances of Mycorrhizal Fungi

Every species has an optimal set of environmental conditions under which it will grow most efficiently and produce the most offspring. Different fungi, like plants, have different niches and thus physiological characteristics (Figure 7.3). While we cannot always differentiate between the absences of fungus and its inability to colonize a susceptible host, environmental factors can indeed control both fungal survivorship and the ability to colonize susceptible hosts (Marx et al., 1970; Bougher and Malajczuk, 1990; Thomson et al., 1994). Accordingly, we address the impacts of environmental heterogeneity on the occurrence of mycorrhizae or root colonization in successional environments.

Ecosystem-level disturbances often result in dramatic environmental heterogeneity. For example, the secondary successional environment of a terminal moraine in a receding glacier foreland can be adjacent to a primary successional ecosystem limited in organic resources. Similarly, areas affected by volcanic eruption or fires co-occur with undisturbed areas to create a mosaic of physically and chemically contrasting habitats across the landscape. Although the disturbance regime typically defines the character of a successional environment, there is also substantial variation in the environmental conditions within those disturbed environments. For instance, soil organic matter and nitrogen concentrations in glacier forefront systems tend to increase with time since deglaciation (Matthews, 1992; Jumpponen et al., 1998; Ohtonen et al., 1999). Likewise, the established vegetation patches in these environments also provide local, relatively enriched resource patches (Jumpponen et al., 1998; Ohtonen et al., 1999). Although extremes of resource availability such as these are largely absent in secondary successional systems, there are small-scale disturbances that alter the distribution of nutrients and inoculum. Postfire litter patches contain higher N, P, K, and water availability than the adjacent (bare) soil patches.

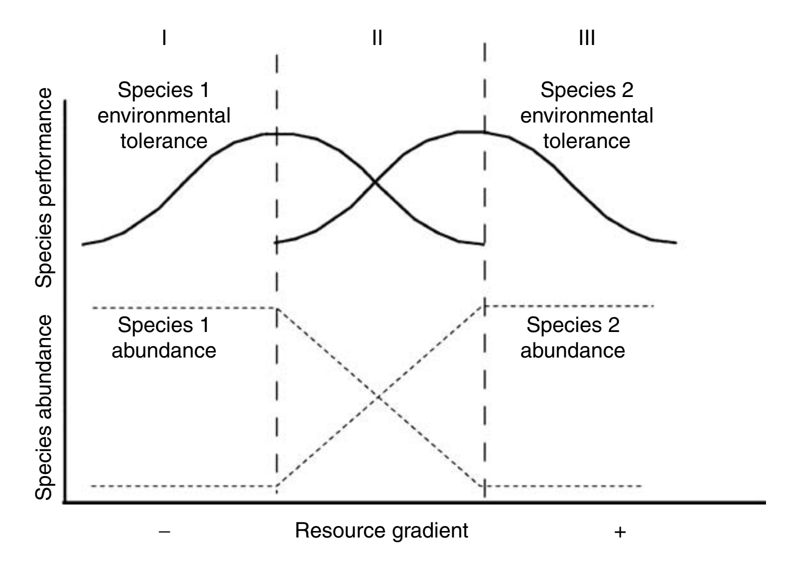


Figure 7.3 Schematic of two mycorrhizal species' environmental tolerances and predicted outcome on their relative abundance. Species performance refers to yield along a resource gradient. The performance curves for the two species outline the use of one resource (fundamental niche) while other parameters are maintained optimal. In region I, species 1 occurs alone because available resources are outside the resource use ability for species 2. In region II, species 1 and 2 co-occur because their resource use abilities overlap in this region. In region III, species 2 occurs alone because available resources are outside the resource use ability for species 1.

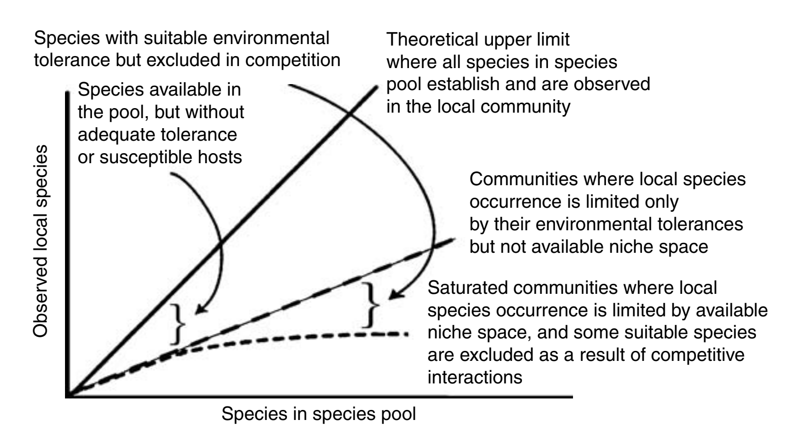


Figure 7.4 The relation between the observed number of species and the size of the available species pool. (Adapted from Connell and Lawton, *J. Anim. Ecol.*, 61, 1–12, 1992.) Note that at low levels of diversity (early succession), all species with adequate environmental tolerances can establish. Only after a large enough pool of species has established do competitive interactions remove species from this pool.

Digging in and redistribution of soil by pocket gophers can either accumulate or reduce mycorrhizal inoculum (Allen, 1988). Ants also concentrate inoculum and nutrients by weaving colonized roots into their seed-caching areas of the mound. All of these examples underline the need to take environmental factors (and biotic interactions) into effect to correctly interpret mycorrhizal community structure, composition, and succession.

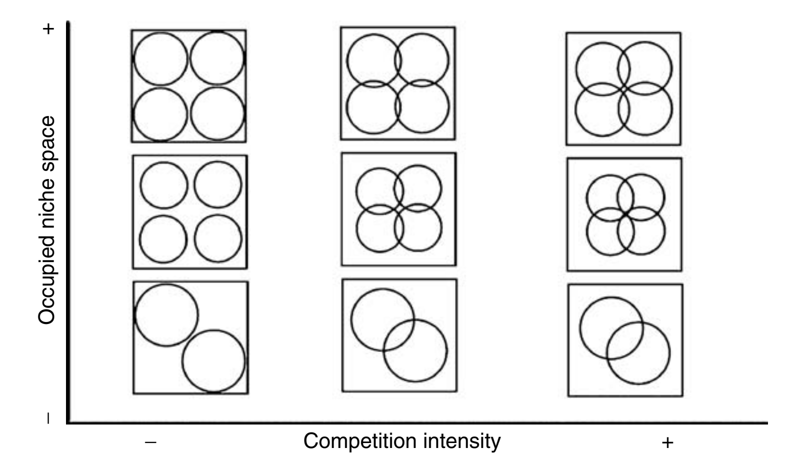


Figure 7.5 Schematic of niche filling and competition intensity. Boxes aligning on a given level of competition intensity share similar levels of competition, while boxes aligning on the niche space axis share similar occupied niche space. Note that niche overlap, not number of species or total occupied niche space, determines competition intensity.

Differences in optima and tolerance ranges (Figure 7.3) for soil abiotic parameters such as temperature or moisture content may be at least partially responsible for filtering mycorrhizal species phenology, dominance, and community composition (Bruns, 1995; Pringle and Bever, 2002). Likewise, segregation of fungal taxa in different forest soil microhabitats has been explained in terms of their diverging preferences for soil organic matter content, moisture, pH, or fertility levels (Johnson and Wedin, 1997; Goodman and Trofymow, 1998; Erland and Taylor, 2002; Neville et al., 2002). Spatial, temporal, and chemical heterogeneity in soil resources can significantly influence mycorrhizal community composition (reviewed in Taylor, 2002). However, little effort and emphasis has been dedicated toward identifying which environmental variables are crucial to defining fungal communities.

Studies in Swedish beech forests identified base saturation and pH and organic matter content as the dominant parameters in determining macrofungal community compositions (Hansen, 1988, 1989; Hansen and Tyler, 1992). Similarly, a large-scale survey of sequestrate fungi (false truffles) in southeastern Australia identified climatic variables, such as moisture availability and temperature, to be important explanatory variables at a landscape scale (Claridge et al., 2000). At a local scale, topographic position, soil fertility, and time since last fire disturbance, as well as microhabitat structures, including leaf litter layer and amount of coarse woody debris, influenced the distribution of sequestrate fungi (Claridge et al., 2000). Studies such as those cited here allow preliminary assessment of the environmental ranges within which fungal species may occur. More importantly, they allow identification of those environmental parameters that may explain the presence of a given fungal species in one environment but its absence in another. For example, Claridge et al. (2000) found that a commonly occurring taxon, *Cortinarius globuliformis*, occurred more frequently in environments with cold temperatures, high moisture availability, and extended periods between fire disturbances. Accordingly, *C. globuliformis* could be identified as a taxon with preference for stable, late successional environments in montane regions. In contrast, *Hymenogaster levisporus* occurred more frequently in environments with reasonably low moisture availability and thin litter layer. Accordingly, extrapolation from these data would identify *H. levisporus* as a taxon with preference for poorly

developed soils — possibly early successional — with little litter in environments that receive limited precipitation.

Nitrogen is also an important driver for fungal community composition (Franklin and MacMahon, 2000), and often the most limiting resource for primary productivity in many terrestrial ecosystems (Vitousek et al., 1997). Primary successional environments, particularly during the early seral stages, have extremely low nitrogen levels, usually in the mineral form. Such conditions likely select for early colonizers such as *Laccaria laccata* (Carpenter et al., 1987) that appear to primarily use inorganic N (reviewed in Smith and Read, 1997). Both descriptive and experimental N enrichment studies also illustrate some of the differences in nitrogen tolerance, acquisition, and utilization among mycorrhizal fungi (Sawyer et al., 2003a, 2003b). For example, an increase in nitrogen availability leads to shifts in EM community composition in both coniferous (e.g., Kåren and Nylund, 1997; Peter et al., 2001) and deciduous forests (Baxter et al., 1999; Taylor et al., 2000; Avis et al., 2003). Lilleskov et al. (2002a) identified both nitrophilic and nitrophobic EM species from an anthropogenic nitrogen deposition gradient in Alaska. *Ampihinema byssoides* and species of *Cortinarius* and *Piloderma* were nitrophobic, and thus abundant in sites with low nitrogen availability. Conversely, *Tomentella sublilacina* and *Thelephora terrestris* were considered nitrophilic and tended to dominate sites with higher overall nutrient availability. Observational studies such as these, however, are often unable to identify the causal factor(s) associated with such shifts in the fungal community. Nevertheless, Avis et al. (2003) showed that when limitations by nutrients other than N were largely controlled, the most substantial differences in EM communities tended to be imposed by N enrichment. Species of EM fungi that differed in their response to nitrogen enrichment also differed in their use of different nitrogen sources in axenic culture (Lilleskov et al., 2002b). Fungal taxa that were common in low-nutrient environments manifested a greater ability to use complex nitrogen sources than isolates from nitrogen-rich environments, indicating adaptation to prevailing conditions. Indeed, EM sporocarps associated with a single (unfertilized) oak stand had $\delta^{15}N$ values ranging from +2 to +11 (A.E. Lindahl and M.F. Allen, unpublished data), because different species of fungi acquired N from different sources: certain EM taxa (*Hebeloma crustuliniforme*) acquire organic N from litter, whereas other taxa (*Pisolithus tinctorius*) acquire inorganic sources of N (Chalot and Brun, 1998).

Similarly, AM communities have been shown to be responsive to environmental parameters, although studies focusing on the environmental control of their community composition are few. As with EM, differences among AM communities may reflect variations in soil moisture, temperature, and pH, which are known to influence AM sporulation (Porter et al., 1987; Cuenca and Meneses, 1996). Root colonization by *Glomus intraradices* (Augé, 2001) and *Glomus mosseae* can be influenced by soil temperature or available moisture (Stahl and Christensen, 1991). Further, Husband et al. (2002) found that nonrandom associations between AM fungi and their hosts were site dependent. Changes in the abiotic environment over time also corresponded with changes in AM species dominance and community composition. Such responses in concert with host phenology have also been used to explain temporal variation in AM communities (Lee and Koske, 1994; Eom et al., 2000; Daniell et al., 2001) and the successional recruitment of seedlings (Helgason et al., 2002).

Specific edaphic parameters also influence the incidence, growth, and turnover of AM fungi (Mosse et al., 1981). Johnson (1993) showed that experimental fertilization treatments altered both mycorrhizal community composition and functioning in a Minnesota grassland ecosystem. Similarly, Egerton-Warburton and Allen (2000) found that members of the Gigasporaceae and larger-spored *Glomus* spp. were largely eliminated

with N fertilization or prolonged nitrogen deposition in California shrub lands (largely from NOx, >30 years; Egerton-Warburton et al., 2001). Observational studies have attempted identification of AM fungal environmental preferences. Rathore and Singh (1995) reported a positive correlation between AM spore abundance and soil phosphorus availability, plus a negative correlation between spore abundance and soil clay content. They and others (Jasper et al., 1979; Schultz et al., 2001) also found that intensive P fertilization limited host dependence on mycorrhizal fungi in prairie soils and agricultural plots. This phenomenon may be due to community shifts among AM fungi; both Koske (1981) and Klironomos et al. (1993) were able to correlate the occurrence of individual AM fungal species to soil P content.

Spatial heterogeneity or stratification of resource availability also results in a patchwork of different environments at local scales. These microsites allow mycorrhizal fungi with different environmental tolerances or requirements to occur in close proximity. Both EM and AM community composition can be correlated with soil depth or horizon (Malajczuk and Hingston, 1981; An et al., 1990; Dickie et al., 2002; Rosling et al., 2003) because a number of variables (including O_2 and CO_2 content, pH, temperature, moisture, and competing soil organisms) covary with soil depth (Taylor and Bruns, 1999).

Changes in soil mineralogical properties also create different habitats for fungi. Intensive sampling of EM root tips in seven distinct soil horizons in a boreal forest podzol illustrated that two thirds of EM root tips occurred within the mineral soils and that half of the EM taxa were restricted to mineral soils (Rosling et al., 2003). Fransson et al. (2000) recovered *Cenococcum geophilum* from organic layers, whereas *Tylospora fibrillosa* occurred in mineral soils. In addition, correlations were detected between the occurrence of mycorrhizal fungi and substrate physical (negative — bulk density) and chemical (positive — moisture content, N, N:P, Ca:Mg) properties in a soil-weathered bedrock (to depth of 2 m; Egerton-Warburton et al., 2003). Furthermore, metalliferous soils constitute strong environmental filters for mycorrhizas. Mycorrhizal types, taxa, and isolates can be selected for tolerance to potentially phytotoxic metals, such as Pb, Al, Ni, Cu, and Zn. Isolates of EM (e.g., *Pisolithus*) and AM (e.g., *Glomus*) fungi tolerant of high soil concentrations of heavy metals demonstrated the capacity to grow on media containing high concentrations of metals, in comparison with isolates from noncontaminated sites (Jones and Hutchinson, 1986; Egerton-Warburton and Griffin, 1995). Metal-tolerant fungal isolates also increased the host plant's tolerance to metals within the soil solution (see Meharg and Cairney, 2000a; Hall, 2002).

7.4.2 Environmental Tolerances as Drivers of Successional Community Change

Clearly, various environmental parameters influence occurrence of mycorrhizal fungi in ecosystems at various scales. How is this relevant to the presence of mycorrhizas in successional ecosystems? As we noted previously, primary successional environments provide an elegant study system wherein drastic environmental differences exist between vegetated patches and adjacent interspaces. Levels of nutrients, especially nitrogen, soil organic matter, and moisture, are higher within these vegetated patches than interspaces, whereas soil temperatures are significantly higher in interspaces than vegetated areas. It follows that when mycorrhizal roots of mycorrhizal plants extend beyond the canopy or patch, they experience a very different set of environmental pressures. Furthermore, many environmental parameters change along the successional gradient, such as the chronosequence of a glacier forefront. Accordingly, we propose that these environmental pressures select a different suite of fungi from the propagule bank (Figure 7.4) that, in turn, colonize available and compatible hosts. These selected fungal communities are different at different

stages of a successional gradient as well as on a local scale within and beyond established vegetation (see Trowbridge and Jumpponen, 2004).

Are such environmental effects also likely in secondary successional ecosystems? We use examples of prescribed fire as a disturbance and its effects on the communities of mycorrhizal fungi. Fire is a frequent disturbance event in many shrub and forest ecosystems. However, not all fires are stand replacing. In fact, prescribed burning has been used extensively as a tool to reduce fuel loads. It is difficult to estimate the effects of fire on fungal communities because of the extreme spatial heterogeneity in root-inhabiting fungal communities. Nonetheless, Stendell and coworkers (1999) were able to show that responses of ectomycorrhizal species to fire differed. The abundance and spatial patterning of fungi in the postfire environment provided clues to their survival. First, the most abundant mycorrhizal species colonizing roots in the litter and topmost organic layers in the prefire environment were reduced to low or undetectable levels after fire; many mycorrhizal fungi occupying deeper (mineral) soil horizons were relatively unaffected. Similar patterns have also been observed in AM communities in shrub lands (Egerton-Warburton, unpublished). Second, fungal species were not randomly distributed among seedlings or on different parts of the same root system. Inoculum for each fungal species thus behaves as a point source: spores in an extensive spore bank (*Rhizopogon*), sclerotia (*Cenococcum*), or mycelia from root tips deep within the soil profile (*Russula*) colonize root fragments (Hagerman et al., 1999; Grogan et al., 2000). Fire obviously alters the community profile, and over time, many of these early colonizing fungi will eventually be replaced by the species that previously dominated the forest community.

It remains unclear how important the effects of low-intensity fires might be on mycorrhizal community structure. Studies on the manipulation of litter on forest floor may provide clues toward an understanding of the impacts of changes in fire-related environmental parameters. Baar and de Vries (1995) reported shifts in EM community structure in seedlings following litter addition or removal treatments in Dutch *Pinus sylvestris* stands. Subsequent pure culture studies indicated that the causal factor for the observed community shifts may have been a result of differential growth rates of various EM fungi in the presence of litter or litter extracts (Baar et al., 1994). Taken together, these studies indicate that the changes in environmental parameters, such as presence or absence of litter, are likely to have profound effects on the mycorrhizal community composition even after a relatively low intensity disturbance. Accordingly, we can conclude that the environmental filter is likely to act upon the selection of the successful EM fungi in both primary and secondary successional ecosystems.

7.5 BIOTIC FILTERS

We will briefly discuss herbivory, bacterial fungistasis, and positive interactions among bacteria and mycorrhizal fungi, as well as competitive interactions among mycorrhizal fungi, as possible biotic filters. We stress that it is not just the presence of various biotic interactions, but the differences among the component mycorrhizal species, that are necessary for any of the possible biotic interactions to act as a selective filter that removes species from the available pool in the propagule bank (Figure 7.4 and Figure 7.5).

7.5.1 Herbivory as a Modifier of Fungal Communities

Herbivory acts as a biotic filter through selective grazing of fungal tissues in soil or indirectly via changes in host physiology and carbon allocation because of defoliation by herbivores. These direct and indirect effects of herbivory influence the relative abundances

and community composition of fungi present within the soil. The impacts of herbivory differ greatly between AM and EM fungi. For example, the positive effects of AM fungi on plant species richness tend to disappear when herbivores are present (Eom et al., 2001; Gange and Brown, 2002), and more AM propagules were recovered in plots that were not grazed by ungulates than in grazed plots (Bethlenfalvay and Dakessian, 1984). In addition, microarthropods show preferences for certain fungi, as well as certain species of EM or AM fungi present in the soil (Klironomos et al., 1992; Klironomos and Kendrick, 1996). Chronic herbivory may reduce EM species richness (Gehring and Whitham, 2002), although results and conclusions from different studies may vary greatly (Saikkonen et al., 1998). The changes in mycorrhizal community are possibly based on the selection of fungal species and propagules that are energetically less costly to the plant. Thus, not all mycorrhizal species are equally affected by herbivory. In pinyon pines, the most herbivore resistant trees supported *Tricholoma*, whereas herbivore-susceptible trees supported mainly ascomycetes. These interactions also tend to be cumulative with environmental stress so that any changes in abiotic (soil) factors further alter the mycorrhizal community within the soil (reviewed in Gehring and Whitham, 2002).

7.5.2 Soil Bacterial Fungistasis

Various components of soil microbial communities interact in complex ways (Lockwood, 1977, 1992; Fitter and Garbaye, 1994; Cairney and Meharg, 2002). We will consider separately the positive and negative interactions between soil bacteria and mycorrhizal fungi as well as among mycorrhizal fungi. The interactions considered here are likely to impact the mycorrhizal fungi selected from the soil propagule bank by either inhibition (fungistasis) or facilitation (mycorrhizal helper bacteria) in successional ecosystems. Negative interactions among different soil organisms are likely to result in competitive exclusion by either interference or exploitation, and affect various life stages of mycorrhizal fungi — namely, spore germination and hyphal extension (Lockwood, 1992). We will consider niche overlap in resource use and extrapolate these results to community-level changes in successional environments.

The soil environment often suppresses the germination of fungal spores and growth of mycelia (Lockwood, 1977). This phenomenon, known as fungistasis, has been suggested to protect soil-borne fungi from germinating and initiating growth under unfavorable conditions (Lockwood, 1977). The mechanisms of the soil fungistasis are unclear, but the absence of appropriate environmental stimuli or unfavorable soil physical and chemical conditions are clearly pivotal for determining the initiation or inhibition of fungal growth. Evidence points toward microbial interactions as a possible key mechanism in the inhibition of fungal growth and spore germination in the soil. Two main factors for soil fungistasis have been proposed: (1) competition between soil bacteria and fungi for limiting resources (primarily carbon) in the soil and (2) antagonism by production of antifungal compounds by microbes.

The direct mechanisms behind the bacterial inhibition of fungal activity are debatable. The microbial-induced fungistasis has often been explained by competition between bacteria and fungi for limited carbon supply (de Boer et al., 2003) because the immobilization of available carbon by the bacterial biomass limits spore germination and hyphal extension (Arora et al., 1983; Ho and Ko, 1986; Mondal and Hyakumachi, 1998). Correspondingly, alleviation of the carbon limitation by addition of simple carbon substrates, such as sugars or amino acids, often reduces soil fungistasis. Thus, soil nutrient resource limitations are important in soil fungistasis (Lockwood, 1977). On the other hand, an additional mechanism for soil fungistasis might be the production of antifungal compounds by microbes (Romine and Baker, 1973; Lockwood, 1992; Liebman and Epstein, 1992,

1994). A wide range of soil-inhabiting microbes produce compounds that effectively inhibit the regrowth and extension of fungal hyphae (de Boer et al., 1998, 2003; Burgess et al., 1999; Behal, 2000). It is likely that both competition among bacteria and fungi and the production of fungistatic compounds act synergistically because the soil and rhizosphere environments contain a vast diversity of organisms with different carbon utilization potentials and metabolic pathways (Toyota et al., 2001). Thus, separation of the two different possibilities is difficult if not impossible.

Although most of the studies on soil fungistasis have focused on plant pathogenic fungi and the effects of either bacterial competition or antagonism on their growth, similar mechanisms are likely to influence germination and growth of mycorrhizal fungi. For example, AM spore germination can be stimulated by volatiles from soil-isolated actinomycetes (Carpenter-Boggs et al., 1995). Like many soil-borne fungal pathogens or saprotrophic fungi, however, mycorrhizal fungi are likely to be susceptible to bacterial antifungal compounds, although sensitivity among the fungal taxa may vary substantially (Lockwood, 1977; de Boer et al., 1998). Competition for soil carbon sources between established mycorrhizas and soil bacteria may be of limited importance because mycorrhizal fungi have direct access to host photosynthates. However, a number of EM fungi have been shown to utilize complex detrital substrates (Meharg et al., 1997; Meharg and Cairney, 2000b) and forage in litter (Bending and Read, 1996). Such taxa may be particularly sensitive to competition for available carbon. Bacterial carbon immobilization may also have significant impacts on mycorrhizae if fungal spore germination is indeed stimulated by soluble carbohydrates. Accordingly, we conclude that even in the absence of direct experimental evidence, bacterial fungistasis and different responses among mycorrhizal fungi possibly influence the selection of fungi colonizing plant roots in successional ecoystems.

7.5.3 Positive Interactions among Mycorrhizal Fungi and Bacteria

Ecto- and arbuscular mycorrhizas have been shown to have bacterial associates. For example, bacteria colonize EM mantle and Hartig net, as well as mycelium and fruiting bodies of EM fungi (Danell et al., 1993; Nurmiaho-Lassila et al., 1997; Mogge et al., 2000). Similarly, AM hyphae have been shown to host both superficial and intracellular colonization by bacteria (Bianciotto et al., 1996, 2000). Although these associations are relatively common, their function and significance have remained largely unknown. Some may simply represent an opportunistic colonization of damaged hyphae (Mogge et al., 2000), whereas others may constitute endosymbiotic associations (Bianciotto et al., 1996, 2000). These associations are likely to have a wide variety of impacts on mycorrhizal fungi and their ability to colonize host roots, ranging from positive to negative (Garbaye, 1994). In this section, we concentrate on how bacteria associated with mycorrhizal fungi might influence the process of root colonization and, in turn, influence the community of fungi colonizing roots in successional ecosystems.

Facilitation among rhizosphere organisms is one possibility. For example, bacteria have been reported to adhere superficially or intracellularly to fungal hyphae on root surfaces and in soil, or be generally associated with the rhizosphere of mycorrhizal plants (Bianciotto et al., 1996, 2000; Mogge et al., 2000; Poole et al., 2001; Minerdi et al., 2002). Although AM fungi are exclusively biotrophic, organic matter can facilitate the growth of extramatrical mycelium — a response that has been attributed to bacterial activities within organic matter (Green et al., 1999; Ravnskov et al., 1999). Some bacterial genera have also been shown to stimulate mycorrhizal colonization (Garbaye, 1994; Budi et al., 1999; Poole et al., 2001). Ruiz-Lozano and Bonfante (2001) hypothesized that bacterial associations with AM fungi may positively influence nutrient uptake by the host plant and nutrient transport from the AM fungus to the plant. The possibility of intimate association between

fungi and helper bacteria exists (Garbaye, 1994; Minerdi et al., 2002), but the precise mechanisms of the bacterial stimulation of mycorrhiza formation remain unclear.

Garbaye (1994) hypothesized that the bacteria may either facilitate recognition between the host and mycorrhizal fungus, stimulate propagule germination, or stimulate mycelial growth. The variety of possible largely unknown interactions in the rhizosphere of mycorrhizal plants leaves much to speculation. We propose, nonetheless, that some fungal taxa may benefit more than others. For example, root colonization of various EM plants by *Laccaria laccata* was stimulated by strains of *Pseudomonas fluorescens* (Garbaye and Duponnois, 1992; Duponnois et al., 1993; Dunstan et al., 1998). However, these results are not universally supported (e.g., Duponnois and Plenchette, 2003), suggesting that host species or environmental conditions may impact the EM fungus responses to bacterial facilitation.

7.5.4 Interactions among Mycorrhizal Fungi

In the previous sections, we concentrated on interactions and associations among organisms that fulfill functionally different positions within successional ecosystems. However, the interactions and competition among organisms are likely to intensify the closer their functional and environmental niches are (Figure 7.4 and Figure 7.5). Although saprotrophic and mycorrhizal fungi can have positive and negative interactions (Lindahl et al., 1999; Lindahl, 2000; Cairney and Meharg, 2002), saprobic fungi are unlikely to impact the ability of mycorrhizal fungi to establish or maintain host root colonization. We are not aware of any examples of facilitation among mycorrhizal fungi. In truth, Fleming (1985) observed neither a direct nor an indirect facilitative mechanism between early- and late-stage fungi. We acknowledge, however, that there are only limited data available on a number of candidates for positive interactions in successional communities of mycorrhizal fungi, as well as on the great variety of direct and indirect possible facilitative mechanisms. Given the paucity of available information, in this section we concentrate on resource exploitation and negative interactions among the mycorrhizal fungi. As only few examples of competition among mycorrhizal fungi are available, we will highlight the available examples and extrapolate those results to community dynamics of mycorrhizal fungi.

Several examples for antagonism or competitive exploitation among different types of fungi are available. Janisiewicz (1996) showed that selection of yeasts with carbon and nitrogen utilization abilities (fundamental niche), similar to those of apple-scab-causing *Penicillium expansum*, greatly improved the yeasts' abilities to minimize apple colonization by *P. expansum*. These results were attributed to substantial niche overlap among the organisms and exploitative competition between the biocontrol yeasts and *P. expansum*.

Different types of fungi may colonize roots simultaneously (Jumpponen and Trappe, 1998). For example, AM and EM fungi may colonize roots of various EM hosts (e.g., Harley and Harley, 1987; Cázares and Trappe, 1993; Chen et al., 2000; Egerton-Warburton and Allen, 2001) and establish functional mycorrhizas (Lapeyrie and Chilvers, 1985). AM and EM have been suggested to be negatively associated (Lodge and Wentworth, 1990; Neville et al., 2002) as they appear to occur rarely in the same root segments (Egerton-Warburton and Allen, 2001). It remains unclear whether the observed negative associations among mycorrhizal fungi are due to antagonism or competition, or merely to different environmental tolerances. However, the poor seedling performance in oaks that were highly colonized by AM and EM fungi indicated competition between mycorrhizal types for carbon (Egerton-Warburton and Allen, 2001). Alternatively, as suggested by Chen et al. (2000), there may be a temporal replacement of AM by EM fungi as the plants age (Egerton-Warburton and Allen, 2001), especially if AM fungi are functionally more important in younger than older seedlings. Local microsite characteristics may also be critical

in determining the success of fungal colonization (Wöllecke, 2001). Both AM and EM fungi may possess different environmental tolerances. For example, AM fungi were more likely to colonize sites with low moisture content, whereas EM tended to colonize roots in moister soils (Lodge, 1989).

Competition also can be linked to variation in fungal life history strategies. For instance, the first AM taxa to invade a root is frequently the most abundant colonizer within the root; i.e., "possession is nine tenths of the law" (Hart and Reader, 2002). The fastest AM colonizers (e.g., family Glomaceae) produce the most extensive colonization and fungal biomass within the root, whereas the slower colonizers produce more extensive extraradical biomass (e.g., Gigasporaceae). In this fashion, spatial segregation may occur between individual fungal taxa on a root at scales ranging from microns to millimeters. In addition, ontogenetic shifts in fungal biomass or turnover result in concomitant increases in the quantity of C, N, and P accrued within fungal tissues.

Although multiple fungi simultaneously occur within a single root system and root fragments (Marks and Foster, 1967; Vandenkoornhuyse et al., 2002a, 2002b), their relative efficiencies in retrieving host carbon or the acquisition of mineral nutrients from soil or soil-borne detritus will also determine and govern their respective abilities for hyphal extension, root colonization, and ultimately, dispersal and reproduction. Uncolonized roots may be subject to intense competition among mycorrhizal fungi (Deacon and Fleming, 1992). When Wu and coworkers (1999) studied the competitive interactions of EM fungi (*Pisolithus tinctorius* vs. *Suillus luteus* or unknown mycorrhizal fungus), they observed that *P. tinctorius* was progressively replaced by the unknown EM fungus, although it remained unclear whether *P. tinctorius* was replaced from already colonized root tips. The competitive interactions among mycorrhizal fungi were attributed to differences in relative growth rates among the competing fungal taxa (Wu et al., 1999). Alternatively, the competing species may inhibit mycorrhiza formation or hyphal extension in one another, as has been proposed generally for fungus soil microbe interactions (see above). Because the competitive interactions are likely to occur at a local microscale, it is unlikely that a single fungus would be able to dominate an entire site or even an entire root system. Similarly, if no clear dominance can be established and all competing fungi remain in the root system, multiple, equally competitive fungi may remain in the root system and coexist — a situation called combative deadlock (Cooke and Rayner, 1984).

The outcomes of the competition are not solely determined by the competitive abilities of the component fungi, nor are the competitive abilites static in time or space (Figure 7.4). Environmental heterogeneity and the dynamics of the critical environmental factors can influence the competition among mycorrhizal fungi. Studies of grain storage fungi have indicated that temperature and water, or nutrient availability, are important determinants (Armstrong, 1976; Magan and Lacey, 1984; Marin et al., 1998). Depending on the environmental conditions, different suites of component fungi were able to establish dominance in inoculated grain. Similarly, the outcomes of competition among mycorrhizal fungi will be modified by different environmental conditions that differ in space and time. This is particularly important in successional ecosystems where localized resource patches are distributed along extended (continuous) gradients, which change over time (temporal dynamics in successional environment) and space (space-for-time substitution in successional seres and accumulation of organic resources).

Can a superior competitor outcompete an already established fungus in a root fragment? We speculate that an established fungus can rarely be expelled from a root unless uncolonized space is exposed. If we assume that in successional ecosystems initial colonization is always established by an allochthonous propagule with limited access to host photosynthate by the established mycorrhizal fungi, drastic community changes would

be unlikely. Observations in primary successional ecosystems (Helm et al., 1996; Trowbridge and Jumpponen, 2003) indicate that the entire root systems are rarely completely colonized during the earliest stages of primary succession. Accordingly, although time and order of mycorrhizal fungus arrival may be important in later stages of succession, they are unlikely to exclude establishment and dominance by less competitive fungal species in early succession (see Figure 7.4).

The biotic interactions discussed here operate on various levels in successional ecosystems. Interactions among trophic levels may influence mycorrhizal colonization via facilitative and inhibitory mechanisms acting upon propagule activation, spore germination, or processes involved in early mycorrhiza formation, such as partner recognition. Although experimental evidence for interactions among mycorrhizal fungi is scanty, we propose that at least competition for space, carbon, and mineral nutrients is acting within successional communities. Additional biotic interactions that may be similar to territorial strategies to exclude invading genotypes may also function in the course of successional dynamics. However, these biotic interactions may be of lesser importance in early primary successional environments, where only limited root space is colonized by mycorrhizal fungi. Later in these primary successional systems, and especially in secondary succession and as root space becomes more limiting, the biotic interactions are likely to increase in their relative intensity and importance (Figure 7.4 and Figure 7.5). In other words, competitive interactions are likely to increase over time in successional environments. We hypothesize that competition occurs in various forms, ranging from simple exploitation for space and nutrient resources to complex metabolic and molecular inhibitory effects. The bottom line is that competition intensifies as the overall available niche space becomes more tightly packed (Figure 7.5) and its impacts become more severe over successional time (Figure 7.4). It is most likely that competition operates mainly via differences in fungal growth rates (see Wu et al., 1999) after accounting for realized niches and possible inhibitory interactions among component organisms.

7.6 SYNTHESIS

7.6.1 Synopsis of the Proposed Model for Succession of Mycorrhizal Fungi

We have incorporated general central themes from community ecology into a simple filter model that focuses on mechanisms of successional changes in mycorrhizal communities. This includes both in- and out-of-site propagule availability into a local propagule or available species pool from which various component species are selected (INPUT). We considered the disturbed landscape patches as islands differing distinctly from the surrounding nondisturbed units. Depending on the level to which the local propagule pool was removed by the disturbance (i.e., the disturbance severity), the relative contributions of allochthonous and autochthonous propagule sources would vary (Figure 7.2). The environmental selection criteria (ENVIRONMENTAL filter) are based on the niche theory: any species unable to perform in any given environment would not be included in the active mycorrhizal community even if its propagules were available (Figure 7.3). In our community assembly model, host–fungus compatibility (HOST filter) is an essential selection criterion, especially for more host-specific EM species. Fungi in the local species pool that establish and survive in the environment at any successional stage will, in turn, interact with other components of the soil community and likely compete for limiting resources. At a BIOTIC filter level, these competitive interactions are likely to eliminate the species occurring in the marginal areas of their environmental tolerances (Figure 7.4).

We suggest that the filters we propose allow greater accuracy in identifying critical factors in fungal community dynamics within a general framework for testable hypotheses. Which of the proposed filters act upon the fungal community in any given environment or successional system? One way to visualize the result of mycorrhizal community filtering is to show how different parameters change community composition. For example, one can test hypotheses on whether the community composition from one species pool changes with different levels of soil fertility following a disturbance event.

In our model, mycorrhizal propagules arrive as an allochthonous input (airborne spores, INPUT) at a barren site (no residual organic legacies, primary succession) after the disturbance event. Availability of compatible or physiologically receptive hosts in the pool of host plants will filter out certain mycorrhizal fungi from the species pool (HOST filter), with the remaining species labile within the propagule bank. The dominant mycorrhizal fungi will also likely be generalists with broad host ranges. The fungi establishing from the available propagule bank should show comparatively high (mineral) nutrient uptake and tolerance to various environmental stresses, excluding competition (ENVIRONMENTAL filter). The most abundant fungi will likely be positively correlated with mineral N and negatively correlated with organic N. Biotic interactions (BIOTIC filter) among the various components in the soil microbial assemblies interact with the mycorrhizal fungi either positively or negatively, facilitating establishment of some while inhibiting others. Competition among mycorrhizal fungi will be limited at the initial stages due to the abundant uncolonized root space (Figure 7.4). As a result, the capacity for fungal expansion into neighboring roots will be moderately high. Although competition for root space is low, the dominant mycorrhizal fungi may take long to establish and contribute to the autochthonous propagule bank. Eventual propagule production by the dominant colonizers will shift the relative contributions from allochthonous and autochthonous sources in the propagule bank, providing a feedback mechanism for community change (Figure 7.2). From a successional perspective, these root communities will be far less complex than in adjacent (nondisturbed) communities or secondary successional systems. Subsequent vegetation and mycorrhizal colonization will further modify the soil nutrient status and, thus, result in changes in the ENVIRONMENTAL filter. One item to consider is the persistence (or inertia) of the initial colonizing mycorrhizal taxa and rate of colonization by other mycorrhizal fungi or saprobes (BIOTIC filter). These differences may lead to time lags between the ENVIRONMENTAL and BIOTIC filters.

In summary, the general principle of our proposed model is that species are selected from a larger regional or local species pool (here, a propagule bank) based on their abilities to pass through a series of environmental and biological filters. Only those species that pass through the entire set of filters prevail and establish under a given set of conditions. These component species will also be able to contribute to the local propagule pool via vegetative, mycelial expansion, as well as via deposition of propagules into the local spore bank.

7.6.2 Applicability of the Proposed Model

7.6.2.1 Can These Assembly Rules Be Applied across Ecosystems?

Although data for some components of our proposed conceptual model are minimal, we argue that the proposed model is sufficiently general to allow development of testable hypotheses upon which the search of universal rules of mycorrhizal community assembly in successional ecosystems can be based. Importantly, autochthonous and allochthonous dispersal factors, as well as relative importance of environmental tolerances and competitive interactions, are likely to vary substantially among communities. We are currently unable to make clear predictions on the relative importance of the different components

in our model across different communities in various successional stages. However, we believe that our model serves as a starting point for developing hypotheses on the specific drivers of mycorrhizal community change.

7.6.2.2 On What Scale Is the Proposed Model Applicable?

The components that we have presented and proposed above operate mainly on small, local scales. For example, competitive interactions are likely to take place on the scale of a root fragment, possibly a proportion of the root system. Similarly, resource patches may be very limited in their size, confining the fungal niche to small spatial units. Although the model components may be limited in their scale, the outcomes of the various filters will influence the community composition not only on local, but also on larger, possibly ecosystem, scales. We argue that incorporation of the local and allochthonous propagule production expands the scale of our proposed model. Our aim is to account for both out-of-site and local contributions to the potential species pool in any successional ecosystem or community. The general, underlying question is: What are the component species that comprise mycorrhizal fungus communities in successional ecosystems? If we consider successional ecosystems as islands demarcated by distinct disturbance events within which various environmental pressures and biotic interactions define successful component species, we should be able to use this model to identify controls of community composition on an ecosystem scale.

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8

Fungal Communities: Relation to Resource Succession

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8.1 AN EXAMPLE OF SUBSTRATE SUCCESSION: THE COLONIZATION OF PINE NEEDLES BY FUNGUS FLORA

Perhaps the most widely studied substrate succession was the successive development of fungal strains and other decomposer organisms on the inside of pine needles, from tree foliage to humified litter. Using litter bags and moist chamber cultures, Kendrick and Burges (1962) followed the fate of Scots pine needles (*Pinus sylvestris* L.) over the course of seasons and years and recognized several well-marked steps in the fungal succession. Their study was exemplary inasmuch as they displaced studies on fungal successions from the field of mycology to that of soil ecology. From their results, it appeared that needles, as leaves (Kinkel et al., 1987), were small temporary islands, the inhabitants of which evolved together with their habitat both in space (vertical litter transfer) and time (season, year), while internal resources became progressively depleted. This scheme was reminiscent of patterns and processes observed in the successional development of plant communities by Watt (1947). Other authors described similar sequences in other pine species, pointing out the worldwide occurrence of a low number of more or less pine-specific fungal colonizers succeeding each other in a linear way (Watson et al., 1974; Mitchell and Millar, 1978b; Soma and Saitô, 1979). Other interesting results of pine studies were that the course of fungal succession was strongly influenced by (1) the start of colonization when the needle was still living (Mitchell et al., 1976; Mitchell and Millar, 1978b), (2) the nutrient status of the foliage (Lehmann and Hudson, 1977; Mitchell and Millar, 1978a), and (3) climate (Van Maanen et al., 2000; Gourbière et al., 2001), but did not seem to be affected by surface grazing of the needles (McLean et al., 1996).

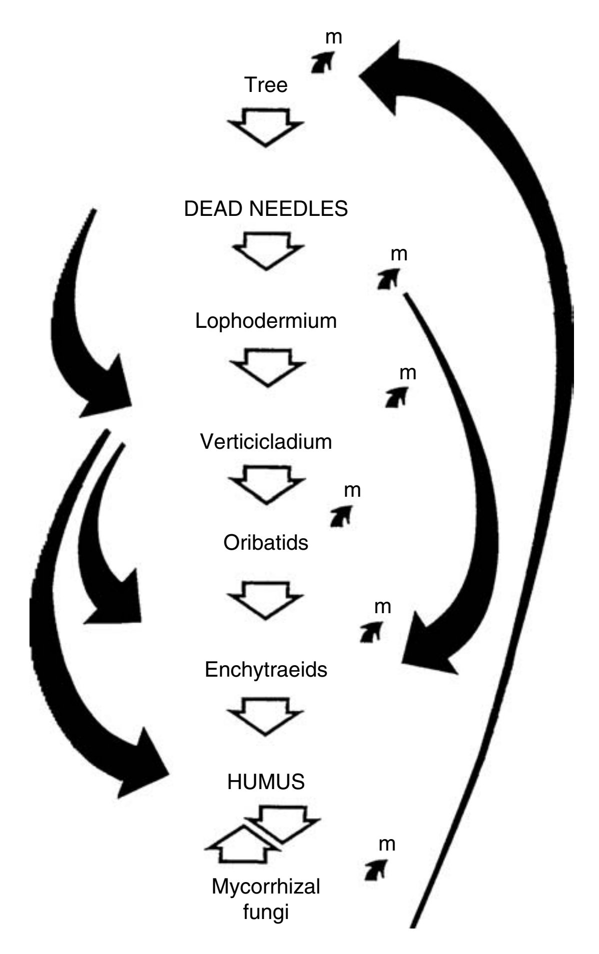


Figure 8.1 Succession of organisms observed during the decomposition of Scots pine needles. m, mineralization. (From Ponge, J.F. (1999). *Going Underground. Ecological Studies in Forest Soils,* Rastin, N., Bauhus, J., Eds., Research Signpost, Trivandrum, India. With permission.)

A more complete pattern, including penetration of pine needles by mycorrhizal fungi and soil micro- and mesofauna, was observed by Ponge (1984, 1985, 1988, 1990, 1991b) by scrutinizing successive layers within a small surface of Scots pine litter (5 × 5 cm). The succession of organisms, both microbial and animal, which took place in pine needles, from death of foliage to disappearance in humus, is summarized in Figure 8.1, taking into account numerous possible shortcuts that were found to occur at the time. It should be highlighted that all organisms involved in this successional course mineralized organic matter through excretory as well as respiratory pathways. In the course of this successional process, which can be considered at first sight a processing chain *sensu* Heard (1994), the substrate was observed to change, as long as resources were exploited by successive inhabitants of pine needles, and part of these resources were lost (Berg and Cortina, 1995) or used for the buildup of microbial and animal biomass (Stark, 1972; Hasegawa and Takeda, 1996).

The exploitation of internal pine needle tissues was found to begin by the use of cell contents, with weak signs of cell wall destruction. Sections of needle parts colonized by *Lophodermium pinastri* (Schrad.) Chev., an ascomyete infecting senescent needles, showed that the fungus was present as hyphae living in the mesophyll tissue, without any

penetration of plant cell walls. In the mesophyll tissue, rows of cells appeared collapsed, without any starch grains, but cells were still entire, although with a distinct browning of their walls. No profound change occurred in the stele, except a distinct browning of phloem cell walls (Ponge, 1984, 1991b). Clearly, the action of the fungus was external and limited to full use of cell wall contents, but browning of cell walls was indicative of its cellulolytic power (Kirk, 1983). All needles colonized by this fungus showed typical black diaphragms delineating territories, each occupied by a clone, and black fruit bodies between the epidermis and the hypodermis. At this stage, entire needles or, more often, needle parts can be occupied by another senescence stage fungus, which fructifies once the needle is on the ground: the coelomycete *Ceuthospora pinastri* (Fr.) Höhn., pycnidial imperfect state of the ascomycete *Phacidium lacerum* Fr., improperly identified as *Fusicoccum bacillare* Saccardo & Penzig by Kendrick and Burges (1962). Some needles, detached from the tree before reaching maturity, were infected by *Lophodermella* spp., a stele-invading pathogenic ascomycete (Williamson et al., 1976; Mitchell et al., 1978).

The second main colonizer, *Verticicladium trifidum* Preuss, conidial state of the ascomycete *Desmazierella acicola* Lib., was resting as small melanized stroma in ostiola of needles colonized by *L. pinastri*. *D. acicola* was also observed to behave as a first colonizer when needles were still not infected at the time they fell on the ground. When needle parts were colonized by *L. pinastri* or *C. pinastri*, while other parts were still not colonized, *V. trifidum* was observed to occur first in fungus-free needle sections, later extending its colonies to the whole needle. In no case were *V. trifidum* and *L. pinastri* found living together in the same section. Whatever happened previously, all needles became progressiveley colonized by *V. trifidum*, and the lower layer of needles was composed entirely of black, softened needles resulting from the activity of this fungus. *V. trifidum* has been shown by Kendrick and Burges (1962) to live several years within the same needle. Several stages were observed during the time this dematiaceous fungus occupied a needle. First, it appeared as thick-walled hyphae growing longitudinally at the inside of resin canals and protoxylem tracheids, but cells from phloem, mesophyll, and transfusion tissues were also penetrated (Ponge, 1984, 1991b). At this stage, the only tissue that remained free of fungus was the metaxylem, but all other lignified tissues (transfusion tissue, protoxylem) remained intact, with transparent and refringent cell walls (except after previous occupation by *L. pinastri*). In some needles, starch grains were still present in mesophyll cells, testifying for *V. trifidum* as a first colonizer. In other needles, previous occupancy by *L. pinastri* or *C. pinastri* was attested to by the presence of hard, recalcitrant tissues, such as diaphragms or pycnidial walls, respectively. At this stage, blackening of the needles was restricted to the vicinity of stomata, where *V. trifidum* filled substomatic chambers with its black stromata. Melanization of pine cells appeared to occur only in stomatal guard cells and nearby hypodermal cells.

The next step was the further development of *V. trifidum*, which formed black stromata in all internal tissues, particularly in the transfusion tissue (Ponge, 1985). Tracheids of the transfusion tissue disappeared progressively by lysis, leaving only areolae visible under the phase-contrast light microscope. Melanization of pine needles affected the entire hypodermis, the cell walls of which appeared covered internally by thick black deposits, despite the absence of fungal penetration. The late development of *V. trifidum* was thus responsible for blackening and softening of pine needles, which made them palatable to soil saprophagous fauna (Hayes, 1963). At this stage, having gained enough energy from the nearly entire consumption of needle internal tissues, this fungus fructified abundantly in the form of dense bushes of black conidiophores protruding from stomatal apertures.

At the stage of the late development of *V. trifidum*, needles were actively penetrated by members of soil mesofauna, particularly oribatid mites and enchytraeids. A succession

was observed from oribatids to enchytraeids, the latter group preferably invading needles that had been previously excavated by oribatids, which filled them with their excrements (Ponge, 1988, 1991b). However, several instances were found of enchytraeids directly penetrating needles previously invaded by *V. trifidum* or even only *L. pinastri* (Ponge, 1984). Defecation by enchytraeids, contrary to oribatid mites, occurred mainly outside pine needles except in most superficial needles, where environmental conditions were probably too dry outside pine needles. Within oribatid feces, pine material appeared to be finely ground by mouth parts of mites and became humified during the intestinal transit, as assessed by optical properties of gut contents. Pine cell walls took a brown and amorphous aspect, with fuzzy contour, indicating strong transformation of both cellulose and lignin (Kilbertus et al., 1976; Saur and Ponge, 1988). Pine material seemed much less transformed in enchytraeid feces, at least when these animals did not reingest oribatid feces. Despite abundance and intense activity, enchytraeid worms contributed poorly to humification, contrary to oribatids; this was also observed by Toutain et al. (1982) in beech litter. Penetration by microfauna (nematodes, amoebae) was observed, using holes made by bigger animals. At this stage a bacterial development was prominent within and around collapsed pine needles, following inoculation with microbes by soil fauna (Macfadyen, 1968; Kilbertus et al., 1976; Touchot et al., 1983). Given the size and shape of the cells, these bacterial colonies were supposed to include nitrogen-fixing strains (Ponge, 1988).

At this stage, needles became highly friable, and most of them were left as small fragments embedded in animal fecal deposits, mostly of enchytraeid origin, which were permeated by dense mycelial webs of mycorrhizal fungi. Dematiaceous (melanized) hyphae of the ascomycete *Cenococcum geophilum* Fr. and hyaline hyphae of the basidiomycete *Hyphodontia* sp. were found to arise from monopodial jet-black and coral-like orange-brown mycorrhizae, respectively. Penetration of remaining needles by *C. geophilum* was prominent (Ponge, 1988, 1990, 1991b), the fungus passing from its aerial to its submerged form, but resources used by this fungus inside pine needles were not identified, although observations on other humus components attested to its chitinolytic and cellulolytic activity. The profuse development of mycorrhizal fungi around and within animal feces and pine needle remains led us to suppose that *C. geophilum* used and translocated nutrients released by microbial and animal activity at the inside of pine needles (Bending and Read, 1995). It should be highlighted that the bacterial development registered before this stage seemed to be arrested by mycorrhizal fungi, maybe under the influence of their antibiotic activity (Krywolap and Casida, 1964; Marx, 1969; Suay et al., 2000).

8.2 STUDIES ON OTHER CONIFEROUS SPECIES

Numerous parallels can be found with studies on other conifers. In particular, we must highlight the paramount work done on fir needles (*Abies alba* Mill.) by Gourbière (1988, 1990), Gourbière and Pépin (1984), Gourbière et al. (1985, 1986, 1987, 1989), Gourbière and Corman (1987), Savoie and Gourbière (1987, 1988, 1989), and Savoie et al. (1990). They described a fungal succession quite similar to that observed on pine needles. Fir needles were first colonized by *Lophodermium piceae* (Fckl.) Höhn., a vicariant of *L. pinastri*, then by *Thysanophora penicilloides* (Roum.) Kendrick, in place of *V. trifidum* in pines. The segregation between *T. penicilloides* and *L. piceae* was similar to that observed between *V. trifidum* and *L. pinastri*. However, a prominent difference was that *V. trifidum* was observed to remain in pine needles for several years (Kendrick and Burges, 1962), which allowed it to exploit most internal resources of decaying needles, while *T. penicilloides* (or *L. piceae* in the absence of further replacement by *T. penicilloides*) was succeeded

within a few months by the white-rot basidiomycete *Marasmius androsaceus* (L.: Fr.) Fr. (Gourbière et al., 1987; Gourbière, 1990). Thus, it did not participate to a great extent in the degradation of cell wall material (Gourbière and Pépin, 1984; Gourbière et al., 1986). The penetration of fir needles by rhizomorphs of *M. androsaceus*, which could occur soon after needle fall, was retarded when needles or parts of needles had been previously colonized by *L. piceae*. This phenomenon was possibly due to the existence of diaphragms, which may act as physical barriers (Ponge, 1984). The presence of *M. androsaceus* has often been recorded in pines, too (Lehmann and Hudson, 1977; Mitchell and Millar, 1978b; Soma and Saitô, 1979; Ponge, 1985, 1991b; Cox et al., 2001), but its presence in coniferous litter seems to be erratic, probably due to the needle-by-needle colonization ability of its rhizomorph system (Macdonald and Cartter, 1961; Gourbière and Corman, 1987). The importance of the time of fall for the colonization of coniferous needles by *M. androsaceus* or other internal fungi (*T. penicilloides* on fir or *V. trifidum* on pine) was suggested by Ponge (1985) and demonstrated experimentally by Gourbière (1990).

8.3 HOW SHOULD OBSERVED SUCCESSIONS BE EXPLAINED?

In the course of the above-mentioned successional processes of coniferous needle decomposition, food and habitat resources for fungi change to a great extent. The exhaustion of cell contents by early colonizers is followed by the differential attack of cellulose-rich and then lignin-rich cell wall material (Savoie and Gourbière, 1988; Cox et al., 2001). In the meantime, fungal and then bacterial biomass is built up, which constitutes a new food resource for further colonizers (Berg and Söderström, 1979). These changes are accompanied by an increase in nitrogen (Berg, 1988; Hasegawa and Takeda, 1996), water (Virzo de Santo et al., 1993), and metal content (Laskowski and Berg, 1993), while fungal metabolism produces organic acids (Takao, 1965; Hintikka et al., 1979; Lapeyrie et al., 1987; Devêvre et al., 1996), melanins (Kuo and Alexander, 1967; Butler et al., 2001), and other metabolites; among them toxins and antibiotics have been widely reported (Wilkins, 1948; Krywolap and Casida, 1964; Land and Hult, 1987; Betina, 1989). Tannins, terpenes, and other secondary metabolites of coniferous litter exert a selective effect on fungal communities (Black and Dix, 1976; Berg et al., 1980; Lindeberg et al., 1980; Lindeberg, 1985), but are progressively degraded by microbial activity (Rai et al., 1988; Lorenz et al., 2000). Thus, the internal biochemical environment of coniferous needles varies to a great extent during decomposition, which may interfere with fungal requirements (Savoie et al., 1990).

The role of fauna should not be neglected either. Needle-consuming animals create cavities (Gourbière et al., 1985; Ponge, 1991b), comminute and humify organic matter (Ponge, 1988, 1991a, 1991b), mobilize nitrogen (Faber, 1991), and inoculate microbes (Pherson, 1980; Ponge, 1984, 1985); thus, they condition the inside of pine needles in a different way than fungi themselves. Still controversial, while highly probable, is the selection role of differential grazing on fungal successions (Newell, 1984; Klironomos et al., 1992; Bengtsson et al., 1993; McLean et al., 1996). On substrates other than coniferous needles, it has been demonstrated that in the absence of soil fauna, the net result of competition between fungal species was a decrease in the weight loss of the decaying substrate (Rayner et al., 1984; Lussenhop and Wicklow, 1985), while the contrary was observed in the presence of grazing fauna (Lussenhop and Wicklow, 1985). It should not be forgotten that pine material is more or less rapidly, but ineluctably, transformed into animal feces, where other microbial successions can be observed (Van der Drift and Witkamp, 1960; Nicholson et al., 1966; Hanlon, 1981; Tajovsky et al., 1992).

We may wonder whether the observed successions are governed by resources, biochemical interference, or other interactions between organisms. More probably, a complex of biological and nonbiological factors is involved in fungal successions on decaying substrates, as this has been demonstrated in wood (Boddy, 2001). Unfortunately, only partial answers can be found in the published literature, given the high degree of specialization now achieved by soil microbiology and the need for sophisticated methods to adequately address mechanisms. However, some experimental and descriptive studies can throw light on the way by which fungal strains are replaced or cohabit in decaying pine needles. Sometimes, it will be necessary to address other fungal successions, such as those prevailing during wood decay (Levy, 1982; Coates and Rayner, 1985; Renvall, 1995; Boddy, 2001; Hendry et al., 2002) if similar mechanisms can be suspected to occur in decaying needles.

The first result we want to underline is that nearly all fungal strains involved in the degradation of forest litter are known to have cellulolytic activities (Hudson, 1971; Savoie and Gourbière, 1989). *In vitro*, microfungi from the phylloplane, generally classified as sugar fungi (Garrett, 1951), also prove able to oxidatively cleave phenolic compounds (Hogg, 1966; Haider and Martin, 1967; Rai et al., 1988). However, we have shown that early colonizers of coniferous needles, such as *Lophodermium* spp., did not attack lignified cell walls (Ponge, 1984; Gourbière et al., 1986), such attack being rather performed slowly by secondary (or late primary) colonizers such as *V. trifidum* and *T. penicilloides* (Gourbière and Pépin, 1984; Ponge, 1988) and, much more rapidly, by nonspecific white rots such as *M. androsaceus* and *Mycena galopus* (Pers.: Fr.) Kummer (Frankland, 1984; Ponge, 1985; Gourbière and Corman, 1987; Gourbière et al., 1987; Cox et al., 2001). Despite differences in fungal enzymic properties, in particular in the possession of phenoloxydases (Kirk, 1983; Hammel, 1997), the segregation of fungal colonies on the same needle (Gourbière, 1988; Ponge, 1991b) and switch-over effects of previous occupants during fungal succession (Gourbière, 1990) point to the importance of biological interactions (Rayner and Webber, 1984; Wicklow, 1986; Boddy, 2000). Most of these interactions are based on the defense of the fungal individualistic territory by short-distance biochemical interference (Rayner and Webber, 1984; Wicklow, 1992) or, in the case of *Lophodermium* diaphragms, by physical barriers (Ponge, 1984).

The nutrient status of coniferous needles may have an impact on the fungal succession, as demonstrated by Lehmann and Hudson (1977) and Mitchell and Millar (1978a): the application of lime or urea to decaying litter favored the more nutrient-demanding ascomycetes (early colonizers) and disfavored less-demanding white-rot basidiomycetes (late colonizers), while the decomposition rate was increased (Sanchez, 2001). This could indicate that early colonizers are potentially able to fulfill the whole decomposition process but lack nutrients to (1) exploit existing resources and (2) antagonize better-equipped fungi. These, especially cellulolytic basidiomycetes, are able to derive micro- and macronutrients from the degradation of recalcitrant compounds such as cell walls and tannin–protein complexes (Saitô, 1965; Entry et al., 1991), starting with the production of low energy-cost oxalic acid, nonenzymatically active during early stages of cellulose degradation (Hintikka, 1970; Schmidt et al., 1981), followed by high energy-cost enzymic production at later stages of degradation (Kirk, 1983; Hammel, 1997).

All of these results point to biological processes as key factors that determine fungal successions at the inside of decaying coniferous needles. Colonization and dispersal are two fundamental steps of the development of fungal communities, at least from the point of view of the individualistic mycelium (Ogawa, 1977; Rayner et al., 1984; Dowson et al., 1986; Dahlberg and Stenlid, 1994; Gourbière and Gourbière, 2002). Intra- and interspecific competition contribute, in turn, to the shape of the community by restricting each

fungus in both space and time (Rayner and Webber, 1984; Boddy, 2000, 2001). Such interactive processes, including founder effects, i.e., the advantage given to the first invader, have been demonstrated to play an important role in plant successions (Connell and Slatyer, 1977; Finegan, 1984; Grime, 1987; Pickett et al., 1987; McCook, 1994) as well as in fungal successions (Tribe, 1966; Coates and Rayner, 1985; Frankland, 1992; Niemelä et al., 1995; Renvall, 1995; Hendry et al., 2002). Gourbière et al. (1999) modeled the persistence and extinction of a fungal species colonizing a number of discrete resource units and applied this model to the experimental colonization of fir needles. Their results showed that the model, the parameters of which were determined by the experiment, accounted for the observed distribution of needles colonized in the field by the same fungus. Later on, they extended their model to two competing species, demonstrating that both species could coexist even in the absence of any trade-off between competitive and colonization abilities, but that the outcome of competition depended on a founder effect (Gourbière and Gourbière, 2002). Recent discoveries did not prove unequivocally that biological traits of individuals/species and their interactions are the only reasons for fungal successions, but rather that biological patterns and processes play a decisive role in the way by which species are assembled in both space and time, as this has been recognized for a long time in plant communities (Watt, 1947).

8.4 CONCLUSION: A STORY OF CONIFEROUS NEEDLES

A hypothetical scheme that explains most of the variation observed in the fungal colonization of coniferous needles can be drawn on the basis of present knowledge. Colonization of the inside of needles starts by the penetration of a restricted array of fungal strains that are able to withstand the biochemical environment of coniferous foliage (phenols, terpenes, carbon dioxide). This early colonization occurs while needles are still attached to the tree, during the senescence stage. This step can be precociously achieved when fungal pathogens penetrate the needle, which causes its premature fall. Once the needle has fallen on the ground, the development of these early colonizers goes further, at the inside of territories delineated by barriers (biochemical, physical) created by each individualistic mycelium, until reproduction organs are produced. As far as original toxic compounds are degraded and fungal defenses are alleviated (for instance, following full use of energy for fructification), colonization may progress through the development of other, less specialized strains already present as resting organs at the needle surface or able to transport energy from needle to needle through rhizomorphs. Litter-dwelling animals play an active role in the dissemination of fungal spores and possibly, if not clearly demonstrated, by stimulating or impeding the development of some fungal strains, according to their feeding preferences.

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9

Emerging Perspectives on the Ecological Roles of Endophytic Fungi in Tropical Plants

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9.1 INTRODUCTION

Fungal endophytes live inside of plant tissues (e.g., roots, stems, leaves) without causing apparent harm to their host. Although the definition of the term *endophyte* is a matter of debate (Wilson, 1995a), throughout this chapter we refer to endophytes as those fungi that live inside of foliar plant tissue, excluding a discussion of stem- (see Evans et al., 2003) and root-associated (i.e., mycorrhizal) fungi. Foliar endophytes are highly diverse and have been documented in nearly all plants sampled (e.g., mosses, liverworts, ferns, conifers, and angiosperms; see Carroll, 1988; Clay, 1988; Petrini, 1991; Arnold et al., 2000, 2003; Arnold, 2002; Davis et al., 2003). Despite the growing recognition of their occurrence among species representing many plant lineages, their ecological roles are still poorly understood.

The best-studied endophytes are Ascomycetes, belonging to the family Clavicipitaceae. These fungi are found growing systemically in the aboveground tissues of some temperate grass species (e.g., *Festuca* spp.; see Saikkonen et al., 1998; Clay and Schardl,

2002). Infected plants often harbor a single fungal genotype, and asexual endophytes are typically transmitted vertically from maternal plants to their offspring via seeds. Endophytes associated with some domesticated grasses are generally thought to act as mutualistic symbionts (see Clay, 1991; Clay and Schardl, 2002; see also Faeth, 2002). These endophytes, which are intimately associated with their hosts, can confer an array of benefits upon their hosts, including tolerance to heavy metals, increased drought resistance, reduced herbivory, defense against pathogens, and enhanced growth and competitive ability (reviewed by Saikkonen et al., 1998). However, vertical transmission, high specificity, and low within-host fungal diversity appear to represent a special case that does not provide a general model for the majority of host–endophyte associations (Saikkonen et al., 1998; Stone et al., 2000; Faeth and Fagan, 2002).

Whether endophytes of woody angiosperms also confer benefits to their hosts is a subject of current debate. While studies with temperate-zone trees show that in some cases endophyte densities are negatively correlated with herbivores and galling insects (Wilson and Carroll, 1994, 1997; Wilson, 1995b; Gange, 1996; Preszler et al., 1996; Wilson and Faeth, 2001), some authors have argued that defensive mutualisms between endophytes and woody plants are likely to be rare (see Carroll, 1986, 1991; Faeth, 2002). In particular, it has been suggested that herbivorous insects may actually promote endophyte infection via folivory, especially in the case of leaf-mining insects (Faeth and Hammon, 1997; Faeth, 2002). However, considering that endophytes are symbionts that obtain resources from and grow within their hosts, it is highly plausible that endophytes of woody plants have evolved ways to defend their hosts, and thus themselves, from being eaten by herbivores or damaged by pathogens (see Frank, 1996; Herre et al., 1999; Arnold, 2002).

Despite this intriguing possibility for mutualistic interactions between endophytes and their hosts, endophyte research in tropical areas has generally been limited to describing the endophyte species found on particular host plants (e.g., Lodge et al., 1996, Bayman et al., 1998; Rajagopal and Suryanarayanan, 2000). Recent studies in tropical areas have demonstrated that endophytes can be extremely diverse within host plants, even within a single leaf. For example, tropical endophytes represent at least five classes of Ascomycota, with 3 to 20 species often coexisting as highly localized infections within individual leaves (Lodge et al., 1996; Arnold et al., 2000). However, compared with endophyte–grass systems, the ecological roles of endophytic fungi associated with leaves of tropical woody plants are poorly known. Only a few recent studies have focused on the basic ecology of these fungi and their interactions with hosts (Fröhlich and Hyde, 1999; Arnold et al., 2001, 2003; Arnold and Herre, 2003; Suryanarayanan et al., 2003).

In contrast to vertical transmission of endophytes in grasses, endophytes associated with foliage of tropical woody plants appear to be predominantly transmitted horizontally via sporefall (Bayman et al., 1998; Lebrón et al., 2001; Arnold and Herre, 2003; Mejia et al., 2003). Leaves are flushed endophyte-free, and then shortly after emergence, they become densely infected with endophytes. There is some evidence to suggest that insect folivory may influence the abundance and diversity of endophytes (A.E. Arnold, unpublished), but the majority of endophyte infections occur without leaf damage as a precursor. Recent studies indicate that young leaves accumulate endophytes shortly after emergence via epiphytic germination of fungal propagules, which then infect leaves via cuticular penetration or growth through stomates (Arnold and Herre, 2003, Mejia et al., 2003).

We have conducted multiyear surveys of endophytes that are associated with *Theobroma cacao* (Malvaceae) and several other plant hosts in Panama. We outline our major findings on diversity, host affinity, transmission, interactions, and pathogen resistance in Table 9.1. Additionally, we discuss the following unanswered questions: (1) Which fungal species occupy which hosts? (2) What is the mechanism for differential host affinity? (3)

Table 9.1 Summary of Selected Findings from Field Surveys and Experimental Work on Endophytic Fungi

Major Findings
1. Endophytic fungal (EF) diversity is extremely high within a single host species. In a sample of 126 *T. cacao* leaves (32 mm^2 of tissue sampled per leaf), 1172 isolates representing 344 morphotaxa were recovered. Within that sample, 20 morphotaxa accounted for roughly 60% of all isolates, with most morphotaxa found only rarely (Arnold et al., 2003). This result is consistent with surveys of endophyte diversity in other families of tropical woody plants (Fröhlich and Hyde, 1999; Arnold et al., 2000; Arnold, 2002), and the prevalence of rare morphotaxa reflects a general pattern among tropical plant-associated fungi (see Gilbert, 2002, Gilbert and Sousa, 2002, Gilbert et al., 2002).
2. EF communities exhibit considerable heterogeneity at small and large spatial scales (Bayman et al., 1998; Arnold et al., 2000, 2003). Although the aggregate fungal communities found on conspecific trees growing within 50 km of each other show relatively high Morsita–Horn similarity (>0.65), that similarity drops off sharply with larger distances (see also Fröhlich and Hyde, 1999).
3. EF transmission is horizontal (among hosts) rather than vertical. Leaves are flushed endophyte-free, and EF are acquired from the habitat over time (see Arnold and Herre, 2003). Leaves appear to saturate in EF density after roughly 2 to 4 weeks.
4. The species diversity of EF communities within leaves increases up to the point of saturation of EF density, generally at 4 to 8 weeks after leaf flush (Rojas et al., unpublished data).
5. EF exhibit differential host affinity. EF communities associated with different host species show striking differences, even when those species are growing in close proximity (Arnold et al., 2000). Specifically, the EF species that tend to dominate the communities in a given host tend to be rare, if they are found at all, in other hosts (Arnold et al., 2003; Herre et al., unpublished).
6. EF growth *in vitro* is strongly affected by the medium. Generally, EF that are commonly found in a given host usually grow best in media that contain extracts of that host species (Arnold and Herre, 2003; Arnold et al., 2003).
7. EF species show a range of dominance interactions *in vitro*, ranging from indifference to active inhibition (Herre et al., unpublished data). The outcome of interactions between any two EF species depends on the medium (Arnold et al., 2003). EF species that commonly occur on a given host generally tend to dominate interactions with more rarely occurring species when tested on medium containing extracts of that host.
8. Hosts with EF-free leaves can be produced by preventing freshly flushed leaves from surface wetting, which is conducive to spore germination and subsequent hyphal infection (Arnold and Herre, 2003). Selected EF can be introduced into leaves in order to conduct experimental tests of the effects of the EF (Arnold et al., 2003, Mejia et al., 2003).
9. Greenhouse trials demonstrate that EF-inoculated leaves resist *Phytophthora* sp. (pathogen) damage, compared with EF-free leaves (Arnold et al., 2003). EF can enhance host antipathogen defenses.
10. Field trials show that EF inoculations can help protect *T. cacao* fruits from loss to pathogen damage (*Phytophthora* sp.) (Mejia et al., 2003, Mejia et al., unpublished).

What is the complete life cycle of the fungi? (4) What is the mechanism of endophyte-mediated host defense?

9.2 QUESTIONS

9.2.1 Which Fungal Species Are in Which Hosts?

Given the diversity of tropical fungi and their hosts, we have not yet begun to scratch the surface of describing how fungi are distributed across hosts. To date, we have isolated endophytic fungi from leaves of eight plant hosts (three vines and five woody plants) in Panama using standard methods (outlined in Arnold et al., 2003). Fungi were grouped to morphotaxa using vegetative features that appeared to conservatively uphold species boundaries as defined by molecular markers (Arnold et al., 2000; Arnold, 2002; Lacap et al., 2003). For the most common and several rare endophytic morphotaxa associated with each host plant species, we used analyses of nrDNA sequence divergence and conducted interaction trials among different isolates to confirm the species boundaries suggested by morphology (see Arnold et al., 2003; Herre et al., unpublished). Further, we used a basic local alignment search tool (BLAST) in order to assign tentative names to the morphospecies (Table 9.2). We emphasize that caution must be used in interpreting the species names given by sequence matches from the BLAST search, primarily due to the incomplete and uneven sampling of taxa in the GenBank database. Therefore, we include the names of our top matches to provide a general idea of the genera and possible species that are commonly found as endophytes in these plants. We note that there is often genetic divergence between isolates that yield the same name as top matches. Given that even small genetic differences can translate to large functional differences (Freeman and Rodriguez, 1993), these observations are consistent with the inference that functional diversity of endophytes is likely to be much greater than the diversity reflected in species names.

To compare differences in host affinity among endophytes, we surveyed and compared the endophytic fungi within two host plant groups. One group consisted of three woody trees on Barro Colorado Island, while the second group consisted of three vines and one woody shrub, all growing in nearby Parque Soberania. Among the endophyte morphotaxa recovered from the trees in the first group (*T. cacao* [Malvaceae], n = 9 leaves; *Heisteria cocinna* [Olacaceae], *Ouratea lucens* [Ochnaceae], n = 3 leaves; Table 9.2), 65.5% were recovered from only one host species (Arnold et al., 2003). Moreover, the most common morphotaxa from one woody host species was usually absent or rare in the other host species. Among the morphotaxa recovered from the second group (*Ipomoea phillomega*, *Ipomoea squamata*, *Merremia umbellata* [Convolvulaceae], n = 16 leaves/host species; *Witheringia solanacea* [Solanaceae], n = 8 leaves; Table 9.2), 75.6% were recovered from only one host species (Van Bael et al., unpublished data). In contrast to the first group, however, several of the most common endophyte–host species were very closely related to the common endophytes in the other host plant species (Table 9.2). This observation of high overlap or similarity among common endophytes in the second group may reflect the relatively higher phylogenetic affinities of these hosts (three Convolvulaceae and one Solanaceae). This raises the question: Do closely related hosts share similar endophytes? A further possibility is that the most common endophytes are more likely to be host generalists, as has been demonstrated for polypores (Gilbert et al., 2002). Further work, in which structured sampling of hosts with different degrees of phylogenetic affinity is done, is needed.

Table 9.2 Species of Endophytic Fungi That Were Frequently Isolated from Leaves of Several Host Plants in Panama

Host Plant Family, Species	Top GenBank Matches[a]
Olacaceae	
Heisteria cocinna	*Guignardia magniferae*
	Xylaria hypoxylon
	Xylaria arbuscula A
Malvaceae	
Theobroma cacao	*Botryosphaeria lutea*[b]
	Colletotrichum gloeosporoides[c] A
	Botryosphaeria dothidea[d] A
	Botryosphaeria dothidea[e] B
	Colletotrichum gloeosporoides[f] B
	Phomopsis sp.
	Colletotrichum gloeosporoides C
	Xylaria longipes A
Ochnaceae	
Ouratea lucens	*Guignardia endophyllicola*
	Phyllosticta sp.
Convolvulaceae[f]	
Ipomoea phillomega	*Glomerella cingulata*[g] A
	Xylaria arbuscula B
Ipomoea squamata	*Glomerella cingulata* B
	Curvularia affinis
	Colletotrichum truncatum A
Merremia umbellata	*Xylaria longipes* B
	Colletotrichum gloeosporoides D
	Colletotrichum truncatum B
Solanaceae[f]	
Witheringia solanacea	*Glomerella cingulata* C
	Colletotrichum truncatum C
Rubiaceae	
Faramea occidentalis	*Xylaria* sp.
	Glomerella cingulata D

Note: Identities are based on BLAST searches of the National Center for Biotechnology Information GenBank database using internal transcribed spacer (ITS) sequences (Altschul et al., 1990).

[a] Listed are the fungal species present in GenBank with which endophytes showed the highest affinity. Letters signify samples that were genetically distinct, despite receiving the same name.

[b–e] Ranking for the most frequently encountered endophyte species in one *T. cacao* collection of 10 leaves (Rojas et al., in preparation).

[f] Identifications represent the two or three most common fungi per plant species in these families.

[g] Note that *C. gloeosporoides* is an anamorph of *G. cingulata*.

9.2.2 What Is the Mechanism for Differential Host Affinity?

In addition to carefully designed surveys of leaves from different species, experiments are important for distinguishing true host affinity from spatial artifacts (i.e., localized dispersal within host crowns) and for examining the mechanisms behind host affinity when it is observed (Arnold et al., 2003). Recent experimental work has demonstrated growth differences among endophyte morphotaxa frequently collected from *T. cacao*, *H. cocinna*, and *O. lucens* when they were plated on separate media containing leaf extracts from each host species. In >75% of the cases, growth rates were higher on media containing extracts of the host species from which they were most frequently isolated in the field surveys (Arnold et al., 2003). Moreover, the growth rates of endophytes *in vitro* (with host plant extracts) corresponded to their relative abundance *in planta*, with common taxa from a given host growing better than rare taxa. In sum, host-specific leaf chemistry appears to favor the growth of some endophytes over others, and highest growth rates are observed when endophytes were cultivated on extracts of the host species for which they displayed highest affinity in the field. By mediating the growth of particular endophyte species, host-specific leaf chemistry may also influence the outcomes of competitive interactions among endophytes or among endophytes, herbivores, and pathogens.

9.2.3 What Is the Life Cycle for Tropical Endophytic Fungi?

Very little work has been done to establish the complete life cycles of the fungal endophytes identified from woody angiosperms. Reproductive structures of some of the fungal associates are readily observed in nature. Fungi typically identified as the most prevalent dicotyledonous taxa (e.g., *Xylaria* spp. and *Colletotrichum* spp.) are also often encountered on the tropical forest floor developing from leaf and wood litter (Bischoff, personal observations). The current dogma is that the fungi contained within the plant reproduce after the plant tissue (e.g., leaves and stems) senesces or abscises (Wilson, 2000). These fruiting structures then provide inocula that lead to new infections of developing leaf and branch tissue (Malloch and Blackwell, 1992).

Although horizontal transmission via spores after leaf senescence is a likely method of dispersal, it is doubtful that it is the only form in which horizontal transmission occurs among the endophytes of woody angiosperms. Species of the grass endophytic genera *Epichloë* and *Balansia* are known to vertically transmit by systemic infection of the host embryo (Freeman, 1902; Clay, 1986). In contrast to these clavicipitaceous endophytes, there has been little evidence of vertical transmission among endophytes of woody dicots. As in previous studies (Bayman et al., 1998; Lebrón et al., 2001), we have observed that seedlings at germination and leaves at emergence lack cultivable endophytes. However, endophytic species have been found associated with host seeds while attached to the parent plant (Petrini et al., 1992; Wilson and Carroll, 1994). These fungi may then disperse with the angiosperm seed, sporulate, and thus provide the inoculum for the newly established seedling. This would help maintain a host–symbiont relationship even in founder events of dispersal. Grass endophytes living asymptomatically in plant tissue were discovered over 100 years ago (Vogl, 1898). Despite extensive work focused on this plant–host interaction over the ensuing years, it was not until 1996 that *Neotyphodium* sp. (the anamorph of *Epichloë*) was found to develop a mycelial net and conidiogenous cells along the leaf surface of *Agrostis hiemalis* and *Poa rigidifolia* (White et al., 1996). The authors determined that the epiphyllous conidia are likely responsible for some of the horizontal transmission occurring in the grass–*Epichloë* interaction. It is possible that this inconspicuous mode of dispersal is also occurring in some of the woody endophytic species.

When discussing the spore dispersal and life cycles of endophytes associated with woody plants, we find that there are more questions than answers. This is especially true

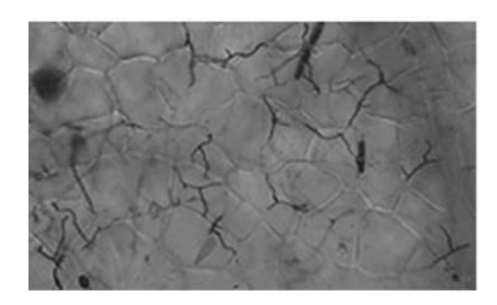
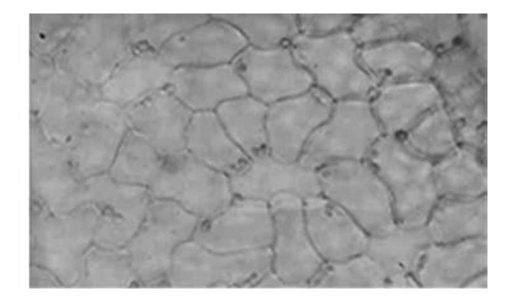



Figure 9.1 On the left is an image of a *T. cacao* leaf with endophytes (E+) that have been introduced experimentally and appear as black lines. On the right is an image of an endophyte-free (E–) *T. cacao* leaf. (Photos by L. Mejia.)

of the tropical woody angiosperms. For example, why fungi wait until senescence to reproduce, what cues their reproduction, and how within-leaf competition influences endophyte fitness require further research. Further, due to the high diversity of these endophytes (Arnold et al., 2000), it is likely that many different types of life cycles will be found among these fungi. For example, many of these endophytes are also regarded as pathogens of particular hosts. It may be that these organisms are able to live in an asymptomatic manner in one host but cause disease in another. Detailed studies of these organisms and their dispersal methods may provide clues to host shifting and the origins of symptomatic pathogens in susceptible hosts.

9.2.4 What Is the Mechanism of Host Defense?

Two recent studies have demonstrated that in at least some cases, endophytes can enhance host defenses against pathogens (Arnold et al., 2003; Mejia et al., 2003).Two key methodological discoveries allowed this work to occur. First, we found that by keeping leaves dry as they grew, the leaves remained endophyte-free (E–) (Arnold, 2002; Arnold and Herre, 2003; Mejia et al., 2003; see also Wilson and Carroll, 1994; Wilson et al., 1997). Second, we were able to introduce endophytes into E– leaves, in combinations and concentrations of our choosing, and thereby create endophyte positive (E+) leaves (Mejia et al., 2003; Arnold et al., 2003). Leaves that were E– and E+ could be generated within individual seedlings of *T. cacao* (Figure 9.1).

In a greenhouse experiment (Arnold et al., 2003), we generated seedlings (n = 70) in which half of the focal leaves were inoculated with a group of seven endophyte species (from the genera *Colletotrichum*, *Xylaria*, and *Nectria/Fusarium*) that had shown previous *in vitro* activity against a foliar pathogen, *Phytophthora* sp. Thus, each seedling contained endophyte treated (E+) and untreated (E–) leaves. Eighteen days after endophyte treatments, we applied a strain of *Phytophthora* sp., isolated previously from symptomatic *T. cacao* in Panama, to a subset of E+ and E– leaves. The final experiment included all factorial combinations of endophyte (E) and pathogen (P) presence and absence. After 15 additional days, we assessed pathogen damage by determining leaf mortality and the area of damage on surviving leaves.

Leaves without endophytes and with *Phytophthora* (E–P+) experienced leaf death and abscission 2.8 times more frequently than did leaves inoculated with endophytes (E+P+). Moreover, on P+ leaves that did survive, necrotic lesions were significantly larger on leaves without endophytes (E–P+) than on leaves with endophytes (E+P+). Although the protection by endophytes was apparently localized to individual leaves, entire host plants were affected by the presence or absence of endophytes. For example, when we

considered both leaf loss and leaf damage on retained leaves, surface area available for photosynthesis decreased by 32.3% for E–P+ treatments relative to E–P–, but only by 14.1% for E+P+ treatments relative to E+P– (Arnold et al., 2003).

While this experiment demonstrated that endophytes limit pathogen damage in *T. cacao*, the mechanism for this defense remains unclear. One clue, however, was the apparent localization of defense to endophyte-infected tissues. This observation, combined with observations of interactions among endophytes *in vitro* (Herre et al., unpublished data), suggested that interspecific interactions among endophytes and pathogens may play an important role in mediating host defense. To explore this hypothesis, we assessed *in vitro* interactions between 50 endophyte morphotaxa isolated from *T. cacao* and three major cacao pathogens (*Phytophthora* sp., *Moniliophthora roreri*, and *Crinipellis perniciosa*; Mejia et al., 2003, unpublished data). In interactions on standard media (2% malt extract agar), 40% of the endophyte morphotaxa appeared to antagonize at least one of the pathogen species, while the remaining endophytes had no effect or were themselves antagonized. Interestingly, when we repeated the interaction trials on media containing leaf extracts of *T. cacao*, the outcomes differed qualitatively and quantitatively. Together, these observations suggest that direct interactions among endophytes and pathogens are complex, diverse, and sensitive to host-specific leaf chemistry. The diversity of endophytes and their interactions may contribute to effective antipathogen defense in woody plants. Because host plants must deal with ever-changing and diverse pathogens in tropical forests, this form of defense is likely to be enhanced when endophytes are highly diverse within and among leaves, plants, and host species.

9.3 CONCLUSIONS

We are only beginning to understand the ecological role of endophytes in natural tropical communities and to realize their applied potential. It is clear that horizontally transmitted endophytes can enhance and supplement host defense against pathogens. The mechanism of defense appears to be in part affected by the outcome of interspecific competition among endophytes and pathogenic fungi, which in turn appears to be influenced by plant chemistry. There are still many outstanding questions about mechanisms of defense and about the potential mutualism between endophytes and their hosts. For example, what are the costs of harboring endophytes to hosts? What is the relative importance of abundance, diversity, and species composition of endophytes in determining whether antipathogen defense occurs? Do endophytes in woody plants provide other types of defense to their hosts, such as against herbivores? An additional obvious need is to expand the work into other host species, in order to assess the generality and frequency of such endophyte-mediated effects.

In addition, the extent to which the interactions among endophytes and their hosts represent true mutualisms deserves further study. In general, mutualistic interactions between hosts and vertically transmitted symbionts can be easily reconciled with existing theory (reviews by Herre et al., 1999; Leigh, 1999). In contrast, horizontally transmitted symbionts are expected to behave less mutualistically and may tend toward antagonism. Nonetheless, several recent examples of horizontally transmitted mutualists, such as pollinators (Herre, 1999), mycorrhizal fungi (Husband et al., 2002), and endophytic fungi, may challenge the existing theory.

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10

Classical Methods and Modern Analysis for Studying Fungal Diversity

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10.1 INTRODUCTION

In this chapter, we examine the use of classical methods to study fungal diversity. Classical methods rely on the direct observation of fungi, rather than sampling fungal DNA. We summarize a wide variety of classical methods, including direct sampling of fungal fruiting bodies, incubation of substrata in moist chambers, culturing of endophytes, and particle plating. We also cite and discuss study designs for documenting diversity and monitoring species, and analytical methods that have been used for data produced using classical methods. Selected examples of such mycological studies are cited so they may serve as models for future research.

The advantages and disadvantages of using classical methods and how these differ from molecular methods are discussed first. Next, we discuss differences in study goals as an important factor when selecting methods for sampling and analyzing fungal diversity. For example, detecting rare or endangered species generally requires different methods from general assessments of fungal diversity or monitoring changes in populations or communities. Selection of appropriate methods for assessing fungal diversity is also highly dependent on the ecological characteristics and distribution or dispersion patterns of the targeted fungal group in both time and space. The types of methods that have been used in classical fungal diversity studies are discussed in the context of the ecological groups for which they are appropriate, and we have cited selected examples in which each of

these has been successfully employed. In the last section, we cover statistical and other analytical methods that can be applied for different goals or hypotheses regarding fungal diversity and conservation.

10.2 ADVANTAGES OF CLASSICAL METHODS

Despite recent advances in the use of molecular methods, there are still many advantages to classical methods for studying fungal diversity. Classical protocols have been developed for studying any substratum or group of fungi and are described in detail in Mueller et al. (2004).

One of the most useful products of a classical study is a list of species found during the study. Due to the relatively small number of species that have been sequenced, it is often impossible for a molecular-based study to present a similar list. Assembling a species list enables researchers to compare data across sites and studies and among different taxonomic or ecological groups. By combining species lists from multiple studies, researchers can determine basic information about individual species, such as geographic range, host relationships, and ecological distribution. The fungal communities of different areas can be compared to determine patterns of species diversity. Additionally, the data from various studies can be combined in order to perform meta-analyses, which can be used to determine the biological and environmental factors that influence fungal community structure at large scales. Classical methods are also the only methods that can be used to demonstrate which fungi are reproducing in a particular environment or on a given substratum, as opposed to which fungi are present but cannot reproduce.

Classical methods are often used to inventory fungi over a clearly defined area or amount of substrate. Researchers often measure environmental variables, such as pH, soil nutrient content, weather-related variables, and biotic variables (e.g., plant community composition or biomass), on the same plots or substrates. Numerous statistical techniques are available to help investigators evaluate the impact that these factors have on fungal communities. Studies using molecular methods can also include environmental measurements, but the data for molecular studies and environmental measurements are often taken at vastly different scales. Combining molecular data from a few grams of substrata with environmental data taken over a much wider area is a major challenge for fungal ecology.

One final advantage of classical methods is that compared with molecular methods, they are generally less expensive and need less specialized equipment. These are important considerations for many investigators, especially those in developing nations.

10.3 DISADVANTAGES OF CLASSICAL METHODS

Despite their widespread use, classical methods have certain disadvantages when compared with sampling using molecular techniques. Some species may not grow or produce reproductive structures in culture and may reproduce rarely in natural settings. These species will be missed by traditional sampling methods, even though they could be important members of the fungal community. The fact that some species will not be detected clearly has the potential to bias classical studies. Unfortunately, it is difficult to assess how many species are missed by classical techniques or to determine if this can bias the results of any particular study. While molecular-based studies of fungal diversity can provide an independent assessment of the fungal community, they are limited to sampling a small area, which can result in a different set of biases.

Compared with molecular techniques, classical sampling methods can be considerably more time consuming. Studies based on macrofungal fruiting bodies have shown that even in areas that have been repeatedly sampled for many years, new species can be found (e.g., Arnolds, 1988; Perini et al., 1989; Tofts and Orton, 1998; Straatsma et al., 2001). Additionally, more taxonomic expertise is required for classical methods than for molecular methods, as all of the species must be identified based on morphological characters. The relative scarcity of trained taxonomists can lengthen the time it takes to identify all of the collections and, thereby, lengthen the time it takes to carry out a study.

10.4 STUDY GOALS

10.4.1 Documenting Diversity

One of the most common reasons to conduct a study of fungi is to document the diversity of species present in a particular area. Diversity studies are sometimes used to document the presence of rare or endangered species, but more commonly to demonstrate that they are indeed rare (e.g., Ing, 1996; Arnolds, 1998, 2001; Otto and Ohenoja, 1998; Courtecuisse, 2001; Molina et al., 2001). General survey and inventory methods are not as efficient as targeted surveys for detecting species that are known to be rare or endangered (see following section; Molina et al., 2001). Quantitative, plot-based diversity studies are also used to provide baseline community data in anticipation of future plant succession, disturbance, or stressors such as climate change and air pollutants (Ing, 1996; Arnolds, 2001). In addition, such studies are used to compare fungal communities in different areas, either to aid in prioritizing conservation decisions (Senn-Irlet, 1998; Courtecuisse, 2001) or to gain insight into what factors influence fungal community composition (Lodge, 1997; Heilmann-Clausen, 2001) and temporal variation in fruiting (Straatsma et al., 2001).

10.4.2 Detecting Rare Species

If certain species are known to be rare or are restricted to limited or endangered habitats such as old-growth forests or unfertilized grasslands, it is often most efficient to use targeted surveys to locate populations of these species or their habitats (Molina et al., 2001; Parmasto, 2001). This generally begins with a survey of previously known localities based on herbarium records and reports (Jalink and Nauta, 2001; Molina et al., 2001). If a species is known or suspected to occur only in a restricted habitat, a search for areas with the same or similar habitats that are then searched for the rare species usually follows (Jalink and Nauta, 2001; Molina et al., 2001; Parmasto, 2001; Rotheroe, 2001). This approach has been referred to as "habitat modeling" by Molina et al. (2001) and gap analysis in conservation literature; it is useful for locating additional populations. Systems for ranking and prioritizing rare and endangered species for conservation purposes, such as Red Data Lists, generally use a combination of criteria that include the number of populations of a species in addition to rarity of occurrence and occurrence in a limited range or in threatened habitats (see Kotiranta, 2001; Molina et al., 2001). Rarity of occurrence and range limits are often established using large-scale mapping and recording programs (Courtecuisse, 1993; Fraiture, 1993; Nauta and Vellinga, 2002). If new sites are discovered to be rich in rare and endangered species, these sites are sometimes inventoried to assess overall fungal diversity.

10.4.3 Monitoring

Recently, there has been interest in monitoring populations of individual fungal species. This can be due to the economic importance of a species or because it is believed to be

in danger of extinction in all or part of its range (Moore et al., 2001). Unfortunately, there is relatively little information on the population dynamics of fungi growing in the wild, which makes it challenging to design a monitoring program. Molina et al. (2001) discuss many of the issues involved in monitoring individual species. They point out that several factors need to be considered in a monitoring program, including establishing clear goals. In the case of rare or poorly known species, populations need to be located before they can be monitored. Due to variations in fruiting, several surveys may be needed each year. For commercially harvested species, monitoring needs to include the amount harvested, length of fruiting season, etc., as well as information on harvesting techniques and land use that may have an impact on fruit body production (Molina et al., 2001). At least two studies (Egli et al., 1990; Norvelle, 1995) have shown that mushroom picking alone does not have an apparent effect on fungal fruiting.

10.5 TYPES OF CLASSICAL METHODS

10.5.1 Opportunistic

Mycologists have traditionally used an opportunistic approach to collecting fruiting bodies of macromycetes, and it is often the most efficient way to record new species in a study area. Typically, this entails collecting fruit bodies that are in good condition and are visible along trails. There are several disadvantages of this method. Data from opportunistic collecting are not easily quantifiable, thus limiting comparisons among areas. This method also requires a highly trained collector who can recognize taxa in the field, but there is also a danger of collector bias affecting the results. Some collectors detect only large or brightly colored fruit bodies, or favor particular groups of fungi because they have developed a search image for them. Furthermore, inconspicuous species and those that are easily confused with more common species are often overlooked (Lodge et al., 2004). Some mycologists and other field biologists have adapted this approach to make the search area quantifiable by using a band-transect method in combination with existing trails. In order to quantify the area searched, the length of the trails must be measured, and only fungi that are found fruiting within a set distance from the trail (e.g., 1 m on either side) are collected or recorded.

10.5.2 Substrate Based

The importance of substratum type cannot be overemphasized in relation to selection of a sampling scheme for quantification of macrofungi. Basidiomycetes and ascomycetes have been found to fruit differentially on different types of substrata or diameter classes of wood, and species rarely fruit on dissimilar substrata (Lodge, 1996; Huhndorf and Lodge, 1997). While some fungi fruit rather dependably, others fruit only sporadically, often requiring surveys lasting a decade or more to be recorded from a particular area (Straatsma et al., 2001). Most surveys therefore employ one or several methods (discussed below) to estimate total species richness in their study area based on a finite number of samples. Many years of surveying will be required if several hyperdiverse communities are combined in a single survey, for example, orb weaving and hunting spiders (Colwell and Coddington, 1994) or fungi occurring on different types of substrata (Lodge et al., 2004). Furthermore, fruiting patterns of fungi differ among substratum types, and the abundance and dispersion of the substrata differ, so the methods used for large woody debris are unlikely to be efficient for fungi on leaf litter or soil and vice versa. If a complete inventory is desired, it is better to use different methods for fungi fruiting on large woody debris vs. small substrata or soil and to treat the data as belonging to separate data sets (Lodge et al., 2004).

Substrate-based sampling methods are used for fungi that occur only on discrete, discontinuous, or patchy resources or are restricted to a particular host. If many resource patches or units occur within a conveniently sized measurement plot, then plot methods may be used. If the patches or substrata of interest are widely dispersed, however, it is more efficient to use a substrate-based method. In addition, relative frequencies of fungal species per substrate unit can be used to directly compare areas that differ in substrate frequency, making substrate-based methods advantageous in such cases. Substrate-based methods are used for fungi occurring on large woody debris or snags (Heilmann-Clausen, 2001), dung (Richardson, 2001; Nyberg and Persson, 2002), fruit (Rogers, 1979; Callan and Carris, 2004), animal corpses (Evans and Samson, 1982, 1984; Sagara, 1995; Hywel-Jones, 1997; Benjamin et al., 2004), and those closely associated with particular animals or plants, including commensals or mutually beneficial symbionts (Rand, 2004; Stone et al., 2004; Summerbell, 2004) and pathogens (Barron, 2004; Callan and Carris, 2004; Summerbell, 2004).

10.5.2.1 Sporocarps on Large Woody Debris

For fungi that fruit on large woody debris, it is often most efficient to use a log-based sampling method (Lodge et al., 2004; Huhndorf et al., 2004). The logs should be classified into diameter and decay classes, tree species (or at least conifers vs. dicotyledonous plants), and whether they are upright, suspended, or on the ground. This allows for selection of several representative logs (replicates) from each type (stratified sampling). A good example of this method was employed by Heilmann-Claussen (2001) in Denmark; in that study, the logs were classified into age classes based on historical aerial photographic records. Logs may be located and quantified per unit area using several different methods, including line transects and the point-quarter method (Lodge et al., 2004).

10.5.2.2 Sporocarps on Leaf Litter, Twigs, and Small Branches

For fungi fruiting on fine debris, it is generally recommended to use a plot-based or band-transect method. While relatively large plots have been used for fungi growing on fine litter (25 to 1000 m^2, 5 to 100 m on a side; e.g., Schmit et al., 1999; Straatsma et al., 2001), it is often more efficient to use smaller, 1 m^2 plots distributed along transect lines (Lodge and Cantrell, 1995; Cantrell, 2004; Lodge et al., 2004). If the fungi to be surveyed are so small or cryptic that they cannot be recognized without the aid of a microscope, for example, small ascomycetes on small wood and leaves, the debris can be collected from a quarter of the area in the plot on each sample date and returned to the laboratory for closer examination (Huhndorf and Lodge, 1997; Cantrell, 2004; Huhndorf et al., 2004; Lodge et al., 2004).

10.5.2.3 Sporocarps on Soil and Ectomycorrhizal Associates of Trees

Saprotrophic fungi that fruit on soil and ectomycorrhizal fungal symbionts of tree roots generally require large plots or survey bands in order to detect a majority of the species that are actually present on the site (O'Dell et al., 1999; Straatsma et al., 2001; Lodge et al., 2004). Some species in these groups fruit only rarely (Lodge, 1996; O'Dell et al., 1999; Schmit et al., 1999), and their occurrence in different plots is often patchy (Schmit et al., 1999; Straatsma et al., 2001). Because ectomycorrhizal fungi are associated with only certain types of trees and shrubs, the distribution of the plant community should also be considered when selecting plots. Forest types are often related to topography, so rectangular plots (e.g., 10×50 m) that are oriented at right angles to the slope are often better than square plots for obtaining a homogeneous sample of the same plant association.

10.5.3 Moist Chambers

Moist chambers are used to stimulate fruit body production on substrata that have been collected from the field (Krug, 2004). This method is most often used for fungi growing on leaves or small woody debris, such as ascomycetes (e.g., Polishook et al., 1996; Rambelli et al., 2004) and slime molds (e.g., Snittler and Stephenson, 2000), and fungi growing on dung (Bills and Polishook, 1993; Rossman et al., 1998; Richardson, 2001; Krug et al., 2004). The substrata are usually placed on moist paper towels in an inflated plastic bag or in a container with a lid. The samples are then examined periodically for 2 to 6 weeks for the presence of fruit bodies.

10.5.4 Culturing

10.5.4.1 Endophytes

Endophytic fungi are those that live inside of live plant parts without causing disease, though some may in fact be latent pathogens or mutualistic symbionts (Carroll, 1988, 1995; Viret and Petrini, 1994). Normally symptomless but fully expanded leaves are collected, but petioles, twigs, branches, and roots have also been studied for endophytes (Carroll, 1988, 1995). Typically, the plant parts are surface sterilized using a 95% ethanol wash followed by immersion for 2 to 5 min in dilute (0.5%) sodium hypochlorite, and a sterile distilled water rinse. Small sections of surface-sterilized plant tissue (smaller pieces are better — Carroll, 1995; Stone et al., 2004; but 1- to 2-mm pieces or segments are often the practical, lower limit) are then placed on agar media containing growth inhibitors in Petri dishes (Lodge et al., 1996). The antibiotics and growth inhibitors prevent bacterial growth and slow the growth of fast-growing endophytes that could otherwise inhibit slower-growing fungi. Typical media include Malt Extract Agar (MEA) with 250 mg/l oxytetracyline (Lodge et al., 1996) and MEA with 35 μm/ml rose bengal, 50 μm/ml streptomycin, and 50 μm/ml chloramphenicol added after autoclaving (Bayman et al., 1997, 1998).

10.5.4.2 Leaf Washes

Leaf washes have been used to study the composition of spores on leaf surfaces. Phylloplane fungi are important in natural biocontrol of pathogens (Bélanger and Avis, 2002; Lindow et al., 2002). Epiphytic fungi, including lichens, can comprise a significant amount of biomass in some ecosystems, and they can play critical roles in food webs and nutrient cycles (Lodge, 1996; Stone et al., 1996; Lindow et al., 2002). Most studies of endophytic and decomposer fungi using the particle filtration method also obtain cultures of phylloplane organisms from surface wash water.

10.5.4.3 Particle Filtration

The particle filtration technique was designed by Bills and Polishook (1994a, b) and Polishook et al. (1996) to eliminate or reduce the number of isolates derived from dormant spores in cultures taken from decomposing plant debris. Thus, cultures derived using this method are primarily of vegetatively active mycelia. The disadvantage of any culture method is that only fungi that are capable of growing in pure culture are detected. According to the method (modified by Bills, 2000), leaf litter is air dried for 3 h before microfungal species are isolated using the particle filtration method, though leaves may be dried for a few weeks, if necessary, with little loss of diversity (Paulus et al., 2003). Pretreatment of the leaf surfaces for 2 min in 0.5% NaOCl, followed by washing with sterile distilled water, was found to be effective in killing surface contaminants without reducing the abundance or diversity of fungi cultured from particles (Paulus et al., 2003).

Air-dried, decomposed leaves are pulverized at high speed and then washed with a stream of sterile distilled water to remove spores. The particles trapped on the 105-μm mesh filter are washed several more times and then plated at several dilutions onto agar media in 90-mm Petri dishes using a flamed, bent glass rod (10 plates each of Malt–Cyclosporin Agar and Bandoni's Medium). This procedure should be carried out in a sterile hood.

At least two types of culture media should be used for the initial dilution plates, Malt–Cycloporin Agar (Malt Yeast Agar [MYA], with 10 mg of Cyclosporin A added when the medium is cool; Polishook et al., 1996) and Bandoni's Medium (4 g of L-sorbose, 0.5 g of yeast extract, and 20 g of agar per liter of distilled water). Fifty milligrams per liter of chlortetracycline and streptomycin sulfate are also added to the Malt–Cyclosporin Agar and Bandoni's Medium when the agar is cool to prevent bacterial growth. All fungi growing from particles should be isolated as they emerge to prevent them from inhibiting the growth of other fungi and to prevent bias. After 1 month of grouth, the fungi can be sorted into morphologically similar species (morphospecies). Fungi growing from the particles can be transferred to Petri dishes or slants with MYA (10 g of malt, 2 g of yeast extract, and 20 g of agar per liter of distilled water). Placing subcultures on additional media, such as oatmeal (OMA), cornmeal (CMA), malt (MA), Potato Dextrose Agar (PDA) (see Rossman et al., 1998), or Potato Carrot Agar (PCA, see Paulus et al., 2003) is useful for separation and identification of strains, especially those that fail to sporulate. Common culture media can be found in Bills and Foster (2004). Autoclaved banana leaves, wheat leaves, or autoclaved leaves of the species from which the fungi were originally isolated can be added to PDA, PCA, or MEA media to promote sporulation.

10.5.5 Area-Based Plots

Area-based plots are one of the most frequently used methods in ecology to quantify the number of species per unit area and to provide a basis for comparing areas using statistical methods. Detailed recommendations for the design of plot-based studies of macrofungi are available elsewhere (Molina et al., 2001; Lodge et al., 2004; Mueller et al., 2004; O'Dell and Lodge, 2004). Comparisons of fungal communities using plot-based methods have been made between areas receiving different treatments (Baar and Kuyper, 1993; Shaw et al., 2003), between areas under consideration for conservation or for inventorying for prioritizing conservation of rare species (Molina et al., 2001), between different plant communities or plant associations (Straatsma et al., 2001), and to study the effects of air pollutants on fungi (e.g., Fellner, 1993). Area-based plots must be used to construct species-area curves (see below).

10.5.6 Transect-Based Methods

Transect-based methods are useful for studying how populations vary along environmental gradients (Van Maanen and Gourbiere, 2000). Data in which a single gradient axis is of interest can be analyzed statistically using logistic regression (Van Maanen and Gourbiere, 2000) or correlation analyses. It does not matter if the gradient axis represents a single environmental gradient (e.g., a moisture gradient) or a complex environmental gradient (e.g., an elevational gradient in which temperature, moisture, and other factors vary in concert). Multivariate analyses, such as correspondence analysis, are often used to elucidate patterns in transect-based studies (see below). Transects have been used to demonstrate the association of ectomycorrhizal fungi with a particular ectomycorrhizal host that had a clumped distribution rather than an environmental gradient (Henkel et al., 2002), and to look at patterns of host specialization in relation to host diversity and dispersion patterns (Gilbert and Sousa, 2002).

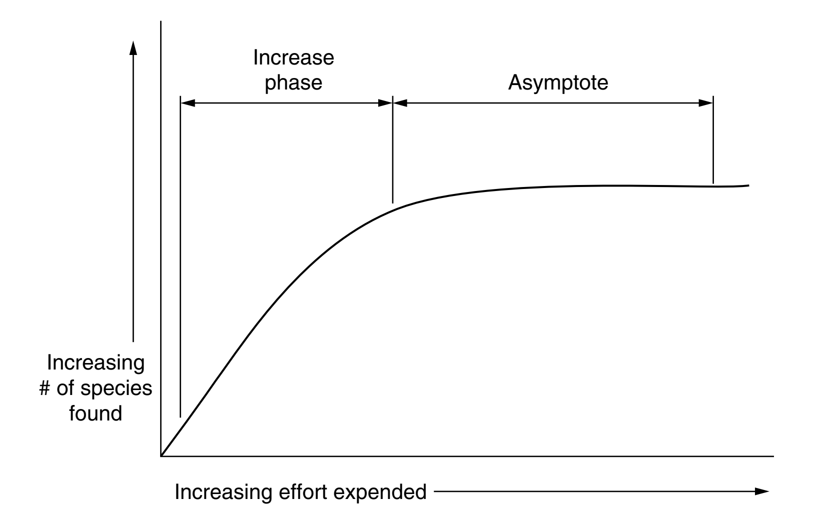


Figure 10.1 An idealized species-accumulation curve. During the increase phase, when little effort has been expended on a survey, increasing effort leads to a substantial increase in the number of species found. Eventually, most of the species are found and the species-accumulation curve reaches an asymptote where more effort results in finding few new species. In practice, few fungal surveys are extensive enough to reach the asymptote.

10.6 STATISTICAL ANALYSES FOR DIVERSITY STUDIES

10.6.1 Species-Accumulation Curves

One of the oldest and most common analyses of diversity data is to construct a species-accumulation curve, such as the familiar species-area curve (Rosenzweig, 1995). A species-accumulation curve is constructed by plotting the cumulative number of species found against some relevant measure of the effort used in finding them. As the cumulative number of species rises, more effort is required to find undiscovered species, which will be reflected by a leveling off in a species-accumulation graph (Figure 10.1). Species-accumulation curves are often used as an aid to determine if sampling effort has been sufficient to discover most of the species present in an area or on a substrate, or to make comparisons between sites.

When constructing a species-accumulation curve, careful consideration must be given to the variable used to measure effort. For a species-accumulation curve to be meaningful, the measurement that is used to quantify effort should be as accurate as the measurement of species richness. Different methods of collecting will give data that are more or less suited to different species-accumulation curves, as we discuss below.

10.6.1.1 Species-Area Curves

Species-area curves are likely the most widely used accumulation curve in diversity studies, particularly in studies that focus on plants. These curves are constructed based on data indicating how many species are found in areas of different sizes. They are often used as a guide to determine if sufficient area has been sampled in a biodiversity study or to determine the size of sample plots that are needed.

Mycologists should be very cautious when using these curves, particularly if they are used to assess sampling effort or predict the total species richness of a given area. When species-area curves are used to determine the species richness of an area, the

underlying assumption is that all, or nearly all, of the species have been discovered in the sampled areas used as data to construct the curve. Regardless of the sampling method used, the high species richness of fungi, their cryptic nature, and their seasonality make it likely that many species in a given area will be missed. Unfortunately, when analyzing species-area curves, it is not possible to distinguish between finding few species due to sampling a small area vs. finding few species because the area studied was not sampled well enough. Therefore, it most instances, using species-area curves will lead to an underestimate of fungal species richness, as many species will be missed in the sampled areas. The underestimation of fungal richness could potentially lead to an overestimate of the adequacy of sampling.

Despite these difficulties, there are some situations where the use of a species-area curve is useful, even if not all species have been sampled. For example, researchers may want to compare the species richness of several different areas but have only diversity data that was collected from plots or transects of different sizes. The species richness of each site can be regressed against the area surveyed (with the variables log transformed, if necessary). Sites can then be compared as to whether they are more or less diverse than would be expected given the area sampled.

A number of species-area curves for fungi have been published. Interestingly, species-area curves constructed from data collected during a single sampling occasion are more likely to level off than those constructed from long-term sampling. Guevara and Dirzo (1998) studied macrofungi in an evergreen cloud forest in Mexico. They did two samplings, one in May and one in September, along two transects. Looking at each transect in each month, they found that once approximately 100 m^2 was sampled, the species-area curve leveled off, but they did not make a species-area curve with the combined data from both months. Brunner et al. (1992) studied macrofungi living in two *Alnus* forests in Alaska by collecting from two 1000 m^2 plots in each forest. After sampling nine times during a single growing season, they determined that surveying 2000 m^2 was not sufficient to fully sample these communities but felt that 3000 m^2 would be sufficient. Bills et al. (1986) inventoried ectomycorrhizal–basidiomycete communities on twelve 256 m^2 plots split between red spruce and hardwood forests in West Virginia. After collecting on approximately 27 occasions over 3 years, they found no leveling off in the species-area curve.

Lodge and Cantrell (1995), working in a tropical forest in Ecuador, surveyed twenty-four 1 m^2 plots divided between two transects for litter-decomposing agarics. Each plot was sampled only once. They found that the species-area curve for these plots leveled off at about 20 m^2. They then looked at the overlap between the two transects. The overlap was approximately 50%, which indicates a good sampling of the community as a whole (Coddington et al., 1991). Cantrell (2004) found similar results in a survey of tropical discomycetes in Puerto Rico and the Dominican Republic.

10.6.1.2 Species-Substrate Unit Curves

Species-substrate unit curves are accumulation curves that use the number of substrate units sampled as a measure of effort. In practice, a wide variety of studies have made use of this type of curve. This can include large substrate units sampled in nature, such as macrofungi on logs (Lindblad, 2001) or the number of substrate units incubated in a laboratory setting (Yanna et al., 2001). Care must be taken to ensure that the unit used to measure effort is appropriate — are the units that are sampled actually equivalent, or do they differ greatly in size and quality? For example, fungi were found to fruit differentially among substratum types and diameter classes of woody substrates in a subtropical wet forest in Puerto Rico (Lodge, 1996; Huhndorf and Lodge, 1997). Even if substrate units

are physically similar, they could differ if sampled at different times of the year or in different habitats.

10.6.1.3 Species-Collection Curves

Species-collection curves plot the number of species found against the number of collections made in order to find them. Collections can be defined as any sample of fungus that can be identified — a fruiting body, an isolate. One of the biggest advantages of constructing a collection curve is that the number of collections processed can be used to determine other useful information. For instance, the monetary cost of processing a single collection could be calculated, and the species-collection curve could be used to determine how much of a monetary investment is required to find a given number of species. Similarly, the amount of time needed to process a collection, or the number of collections that can be found in a single collection trip, can be used to cast the species-accumulation curve in terms of the amount of time that needs to be invested to find a given number of species (Longino and Colwell, 1997).

The biggest drawback to species-collection curves is the need to collect and identify every specimen encountered. To make an accurate curve, every occurrence of every species must be recorded. This can place an extra burden on inventories, as otherwise researchers may not bother to record common species once they have already been found.

10.6.2 Analysis of Species-Accumulation Curves

One of the most common ways of analyzing species-accumulation curves is to inspect the curve to determine if it levels off as effort increases. For example, Lodge and Cantrell (1995) examined a species-area curve to determine that 24 plots of 1 m^2 are sufficient to sample the diversity of agarics living in tropical forest litter. Tofts and Orton (1998) used species-effort curves to determine that 21 visits to a Caledonian pine forest were not sufficient to find all agarics, but they did show a leveling off in the curve when only species that are restricted to Caledonian pinewoods were considered.

Some ecologists recommend using statistical techniques to determine the asymptote of a given curve. Unfortunately, there is no consensus on how this should be done. Several statistical and ecological issues complicate extrapolation. Statistically, estimating diversity from a species-effort curve requires an extrapolation beyond the data (He and Legendre, 1996). To extrapolate beyond the data, it is necessary to choose a particular equation for the species-effort curve. Numerous equations have been proposed (see review in Colwell and Coddington, 1994; Christen and Nakamura, 2000), and different methods may be appropriate for different community structures (Keating et al., 1998). Currently, there is no generally accepted method to determine which species-effort model is appropriate for any given data set.

Extrapolating a species-effort curve has also been questioned on ecological grounds. Extrapolating assumes that given a large enough effort, an asymptote in the species-effort curve would actually be reached (He and Legendre, 1996). However, as sampling effort increases, it becomes more likely that the sampling expands beyond a relatively homogeneous community. This is particularly true for species-area curves, and it has recently been suggested that, in general, species-area curves do not reach an asymptote at scales above 1 ha (Williamson et al., 2001).

The only example of extrapolating of species-area curve for fungi that we are aware of is that of Guevara and Dirzo (1998). They used two equations, the logarithmic and the Clench, to determine if they had sampled sufficient area for macrofungi on two sampling occasions. Both equations gave similar results, indicating that they had collected over 90%

of the species. Unfortunately, they did not continue collecting to determine if the predictions of the equations were accurate.

A more recent use of species-effort curves is to extrapolate the amount of effort needed to find a given number of additional species (Keating et al., 1998). This is similar to predicting the asymptote of a species-effort curve, but rather than the curve being extrapolated to the point where it levels off, it is extrapolated to the point where an arbitrary number of new species have been found. This technique raises the same statistical concerns as determining the asymptote of a species-effort curve. However, if the curve is not being extrapolated very far in comparison with the data collected, the statistical problems are less troubling, as different curve-fitting techniques will probably give similar results.

10.6.3 Nonparametric Species Richness Estimators

In recent years, biostatisticians have realized the limits of species-effort curves to estimate species richness and have worked to develop simpler, more accurate estimators. Numerous nonparametric methods have been designed to do this. These methods are nonparametric in that they do not assume any particular distribution of common and rare species in a community. In general, these methods make use of data on the abundance of each species that has been detected. Depending on the collecting methods used, abundance of species can be measured by number of collections of a species, number of substrate units on which a species is found, number of subplots on which a species is found, number of cultures made of a species, etc. Colwell and Coddington (1994) provide a useful review of many of these estimators. Additional estimators have been derived by Solow and Polasky (1999) and Shen et al. (2003).

Several nonparametric estimators use the total number of species found, the number of species found once, and the number of species found twice to determine the number of species that have yet to be discovered in the area or on the substrate being studied. For example, one of the simpler estimators is $S = S_{obs} + a^2 / 2b$, where S is the estimated number of species, S_{obs} is the number of species observed, a is the number of species found only once, and b is the number of species found exactly two times (Chao, 1984). As the example makes clear, these estimators are attractive to ecologists because they are easy to calculate and rely on information that is easily gleaned from a biodiversity inventory.

Given the large number of available estimators, several researchers have attempted to determine which is the most appropriate for real data sets. Chiarucci et al. (2003) used an extensive data set measuring plant species richness and location from dunes in southwestern Australia. They tested four most commonly used estimators and concluded that none of them performs well: "the estimates obtained can hardly be expected to be accurate and are not likely to be easy to interpret." To provide an accurate estimate, the estimators needed more data than will likely be available and were consistently biased. Chiarucci et al. (2003) also provide a comprehensive review of the literature, testing the performance of nonparametric estimators, which supports their conclusions that the performance of the estimators is disappointing.

The only study that has evaluated these estimators for fungal data sets is that of Schmit et al. (1999). They surveyed macrofungi for 3 years from plots in an oak forest and compared the predicted species richness based on data from the first year to the actual species richness found in the study. Seven species richness estimators were tested, and all of them gave predictions that were consistently too low and that increased as the amount of data increased.

Based on the limited results available, there seem to be two problems with the application of these estimators to fungi or any other hyperdiverse taxonomic group. The first is that more data are required to provide a reliable estimate of species richness than

are generally available in a mycological study (Chiarucci et al., 2003 and references therein). Second, nonparametric estimators assume that the detectability of a species does not change during the time that the study is being conducted. Numerous mycological studies have demonstrated that the abundance of fungal species varies from year to year (e.g., Murakami, 1989; Perini et al., 1989; Schmit et al., 1999), which violates this key assumption. Despite these disappointing results, new methods of estimating species richness are being developed, and new techniques are being used to evaluate them. Undoubtedly, the use of nonparametric estimators will continue to be an active research area that has the potential to provide considerable insight into patterns of species richness of fungi.

10.6.4 Multivariate Methods

In order to understand fungal diversity, it is important to do more than just measure the species richness of fungi at various locations. In recent years, many investigators have worked to identify environmental and biological factors that influence fungal community structure. In practice, the community structure that is examined is either the patterns of presence and absence of species in various sites (= communities) or the patterns in the abundance of species in various sites.

The most important analytical tools for analyzing community structure are multivariate statistics such as cluster analysis and ordination techniques. In general, multivariate methods allow the investigator to group or order a number of sites based on their similarity, which is determined by analyzing a large number of variables. In the context of diversity studies, multivariate analysis is generally used in situations where investigators have information from several sites on the presence or abundance of fungal species, and it sometimes incorporates data on other variables (rainfall, soil chemistry, plant community structure, etc.) as well. In general, these techniques work best when a consistent sampling methodology has been used so that the data from each site are truly comparable. However, multivariate methods can also be used as part of a meta-analysis synthesizing data from several studies, provided due thought is given as to which technique is most appropriate.

A confusing array of multivariate techniques has been developed that can be used to study fungal diversity, and careful thought must be given to choosing the correct one. Luckily, ordination techniques are widely used in the biological sciences, and there is a large statistical literature analyzing the properties of the various techniques. The choice of analysis technique is driven by the data at hand and the goal of the particular study.

10.6.5 Exploratory Analysis

Oftentimes, investigators will wish to use data to group sites, hosts, etc., based on similarities in fungal communities. In some instances, the investigators will have an *a priori* hypothesis about fungal communities they wish to test; methods for doing so are described in Section 10.6.6. This section deals with analytical tools that are useful when investigators are performing an exploratory study and do not have a specific hypothesis to test.

10.6.5.1 Cluster Analysis

One of the most common multivariate techniques is cluster analysis. In cluster analysis, a number of cases, such as sites or hosts, are grouped based on variables such as the presence or absence or abundance of species. Cases that are closely connected on the cluster diagram are the most similar to one another (Figure 10.2). Cluster analysis provides a grouping of cases that is hierarchical, and the analysis shows the places of each case in a series of clusters and subclusters, each more inclusive, but with less overall similarity than the ones below it.

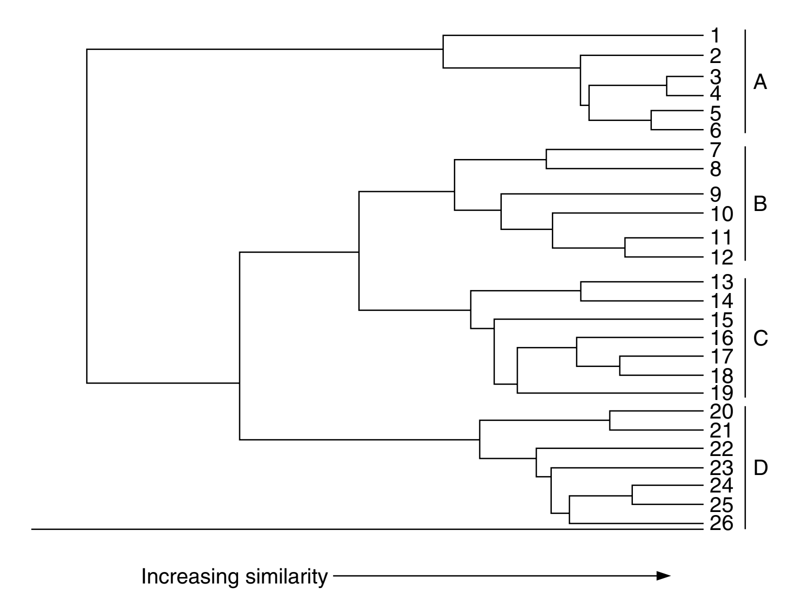


Figure 10.2 An idealized cluster analysis. In this diagram, 26 sites have been clustered based on the similarity of their fungal communities. The closer to the right-hand side of the graph that two communities are linked, the more similar they are. The 26 sites cluster into four major groups (A to D). Group B sites are most similar to group C sites, and group A sites are the least similar to those in the other three groups.

For example, Laganá et al. (1999) used cluster analysis to show the relationship between the macrofungal communities found in Italian forests. They combined data from 30 survey plots in five different forest types and demonstrated that fungal communities sampled from different locations within a single forest type (e.g., evergreen oak wood or chestnut woods) cluster closely together. At a higher level in the hierarchy, the fungal communities from low-elevation deciduous forests clustered together to the exclusion of high-altitude and coniferous forests. Perini et al. (1995) used cluster analysis to compare the plant and macrofungal communities in a number of Italian fir forests. They demonstrated that forests that had similar plant communities also had similar macrofungal communities.

10.6.5.2 Principal Components Analysis

Principal components analysis (PCA) is one of several methods that are used to reduce variation on many axes into a more manageable, smaller set of axes that allows the researcher to more easily visualize the data. Each of the new axes is a linear combination of the original axes. The Euclidean distances among cases and among variables along the new axes are the same as those in the original data set. The scores for the first axis explain the maximum amount of the variability in the data set that can be elucidated by a single such variable. The scores for the second axis explain the maximum amount of the variation that was not elucidated by the first axis; the scores for the third explain the maximum variation that was not elucidated by the first two; and so on for additional axes. Each axis explains less of the overall variation in the original data set than those that preceded it.

In fungal ecology, one use of this technique is to analyze what is known as a site × species matrix. In this type of matrix, the columns are locations where fungi have been

found, such as plots that have been surveyed, host tree species, or experimental treatments. Rows are the species that have been found in at least one site, host, or treatment. Each cell in the matrix is either the abundance of the species or, in the case of a presence–absence matrix, 1 if the species is present and 0 if it is not. PCA is used in cases in which abundance data are available for each species. The axis scores of the sites are then plotted, and sites are grouped by visual inspection.

For example, Newton and Haigh (1998) studied the relationship between ectomycorrhizae and their host in Great Britain. They constructed a matrix with tree genera as the columns and ectomycorrhizal species that associate with the trees for the rows. PCA demonstrated that there were strong similarities in ectomycorrhizal species found on genera in the Fagaceae (*Quercus*, *Fagus*, and *Castanea*), between coniferous genera (*Pinus*, *Picea*) and *Alnus*, *Populus*, and *Salix*.

A second use for PCA is to aid in analyses that include measurements of numerous environmental factors. Oftentimes, when an investigator takes several soil chemistry-, climate-, or vegetation-related measurements at numerous sites, it can be difficult to determine which of those factors influence fungal communities. By using a principal components analysis, the investigator can reduce many environmental variables to a more manageable number of axes (Figure 10.3). A PCA also provides loadings, which tell the investigator the contribution of the variables in the original data set to the axis scores created by the analysis. This can allow an investigator to determine which species or environmental factors in the original data set are most important in differentiating the sites. For example, Hansen (1988) used PCA to study the relationship between soil chemistry and the macrofungal community found on plots in a Swedish beech forest. The study combined a previously published macrofungal survey with measurements of 31 environmental variables in the survey plots. PCA was used to reduce the environmental variables to three principal coordinates that were related to fungal community structure. The analysis showed that saturation of the soil with bases, percent clay in the soil, and percent organic matter in the soil had the biggest impact on the occurrence of macrofungal species.

10.6.5.3 Principal Coordinates Analysis

In usage, principal coordinates analysis (PCO), also known as metric multidimensional scaling, is similar to PCA. Like PCA, PCO reduces the number of variables in a multivariate analysis so that the similarities and the differences between the cases can be more easily visualized. There are several differences between the two analyses, however. One of the most important practical differences is that PCO can use abundance data or presence–absence data. PCO is better than PCA for presence–absence data, as a variety of distances measure can be used, including those designed specifically for presence–absence data. One drawback of PCO is that it does not supply axis loadings, which makes it more difficult to determine which variables have the greatest influence on the results. Peter et al. (2001) used PCO to demonstrate dramatic shifts in macrofungal fruiting in a Norway spruce forest on plots that had been fertilized with nitrogen. Packham et al. (2002) used PCO to demonstrate that the same environmental factors that influence distribution of vascular plants in Tasmanian eucalypt forests also influence the distribution of macrofungal species.

10.6.5.4 Correspondence Analysis

Correspondence analysis (CA) is very similar to PCA and PCO. Unlike PCA, though, correspondence analysis can make use of any type of data, and unlike PCO, the chi-squared distances among the cases and variables from the original data set are preserved in the

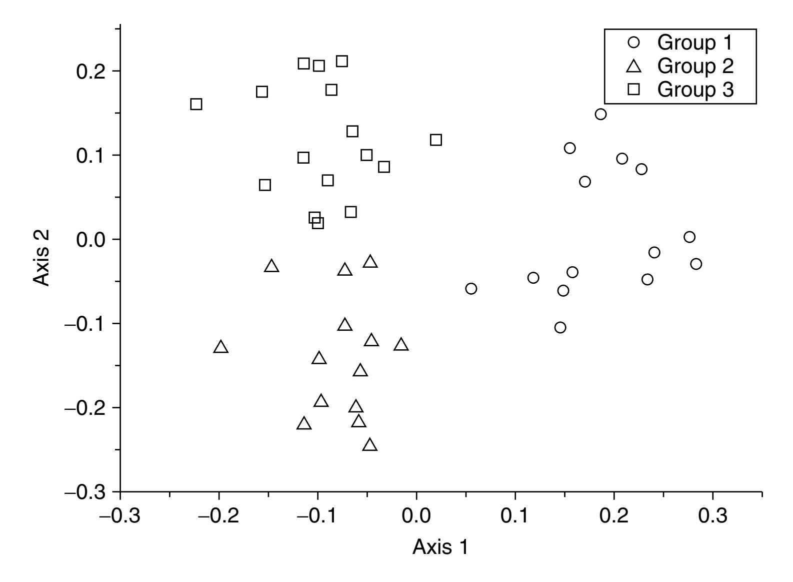


Figure 10.3 An ordination graph from a PCA. In this graph, sites from three different groups (e.g., vegetation types or experimental treatments) are plotted. The axis scores are calculated based on the abundance of each fungal species at each site. The graph shows that the sites from each of the groups cluster together on the graph. Axis 1 separates the sites in group 1 from those of the other two groups, while axis 2 separates the sites in group 2 from those in group 3.

new axes. A frequently used variation on correspondence analysis is detrended correspondence analysis (DCA). Many ordination techniques, including PCA and PCO, are subject to what is referred to as either the arch or horseshoe effect. This is seen when the scores from the first two axes are plotted and the cases show an arch-shaped pattern, which indicates that the two axes are not independent. This is most likely to occur in studies where sampling has been done along an important ecological gradient and there is considerable turnover of species along that gradient. DCA corrects this problem and has become one of the most commonly used ordination techniques in ecology, including fungal ecology (e.g., Termorshuizen, 1991; Høiland and Bendiksen, 1996; Lindblad, 1997; O'Dell et al., 1999; Heilmann-Clausen, 2001; Ferrer and Gilbert, 2003).

Correspondence analysis has shown that different microfungal communities are found on different parts of decaying palm fronds (Yanna et al., 2001). Salerni et al. (2001) used CA to demonstrate that the distribution of macrofungal species in Italian oak forests is influenced by soil moisture and pH.

DCA has been used to examine the factors that influence fungal community structure. Three studies have demonstrated that as logs undergo decay, there is a change in the fungi and myxomycetes that fruit on them (Høiland and Bendiksen, 1996; Lindblad, 1997; Heilmann-Clausen, 2001). It has been shown that there are differences in the communities that decay wood from different host trees (Ferrer and Gilbert, 2003). DCA has also been used to demonstrate that land use (Termorshuizen, 1991) and precipitation and vegetation (O'Dell et al., 1999) influence ectomycorrhizal community structure.

10.6.6 Hypothesis Testing

Ecologists are often faced with a situation in which they have a site by species matrix and data on an environmental factor that they believe will explain the differences in species

presence or abundance between sites. Several ordination methods have been developed that allow researchers to test hypotheses given this type of data. Two of the most commonly used methods are canonical correspondence analysis (CCA) and discriminant function analysis (DFA). Unlike the ordination methods listed above, these two methods are designed to highlight differences in the data among groups or along gradients. CCA is used to determine the relationship between an environmental gradient and community structure. In CCA, the axes are drawn to maximize their correlation with a linear combination of the predictor variables and species data. The output from a CCA can be plotted like that of other ordination techniques, except that in addition to plotting the various sites or species, the environmental variables can also be plotted in the form of arrows that emanate from the origin of the graph. The direction of the arrow indicates a line on the graph along which the variable has its greatest change, and the size of the arrow indicates the magnitude of that change. Kernaghan and Harper (2001) used CCA to demonstrate that elevation has a large impact on the distribution of ectomycorrhizal macrofungi in the Rocky Mountains.

DFA, on the other hand, is used when the sites are believed to fall into distinct categories. The site by species matrix is used in the analysis as well as the *a priori* group designations. The analysis then compares the grouping derived from the data with the *a priori* grouping and determines which sites are classified in the same group by both methods.

10.6.7 Methods to Examine Fungal Distribution

10.6.7.1 Mantel's Test

Oftentimes investigators are interested in determining if differences in community structure between several sites are mirrored by differences in other environmental factors. One way to determine this is to use the Mantel's test. The Mantel's test compares distances between sites measured using one data set with the distances measured using a second data set. The distances used could be a community distance measure, such as the Jaccard Index, physical distance, a distance measure based on climatic data (e.g., difference in rainfall), or distances based on an ordination analysis. The analysis compares the two sets of distances and determines if sites that are distant based on one set of measurements are significantly more distant based on the second set of measurements than would be expected by chance alone. Schmit et al. (1999) used the Mantel's test to provide evidence that at the scale of tens of meters, the closer that two plots are to one another, the greater overlap they have in their macrofungal communities.

10.7 CONCLUSIONS

Classical methods can be used with a wide variety of analytical techniques to characterize communities, test hypotheses, and study the ecology of individual fungal species. Due to the low cost, ease of identifying species, and ability to sample wide areas or many pieces of substrata, classical techniques will remain an important part of fungal ecology. Species lists and environmental information from several classical studies can be combined to analyze fungal distributions at wider spatial scales. However, species that fail to produce reproductive structures are often missed when sampling fungi with classical techniques — a problem not found with molecular techniques.

Combining classical and molecular techniques may allow mycologists to gain a much greater understanding of fungal distribution. In particular, by using these two techniques, it will be possible to compare the species that are merely present in a site or on

a substrata with those that are able to reproduce on that site or substrata. Similarly, it will be possible to compare the geographic or host range over which a fungal species occurs with that over which it reproduces. Comparisons such as these will make it possible to determine the relative importance of different substrates or habitats for the maintenance of fungal populations. It will also be possible to compare the ecological roles played by species that differ in their frequency of reproduction. Studies such as these will provide fascinating new insights into the ecology and population dynamics of fungal species.

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11

Fungal Diversity in Molecular Terms: Profiling, Identification, and Quantification in the Environment

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11.1 INTRODUCTION

Fungi are one of the most phylogenetically and functionally diverse life-forms of terrestrial ecosystems; they are dominant pathogens, symbionts, and decomposers. There may be more than 1.5 million fungal species, of which only 5 to 10% have been identified so far (Hawksworth, 2001). Thus, most fungal diversity is unknown and undescribed. The study of fungal functional diversity, beyond rough classification into trophic categories, remains largely in its infancy. It will grow further through the development of genomics and proteomics on the foundation of molecular phylogenetic diversity that is now being constructed, albeit slowly (Figure 11.1). This review of the study of fungal diversity in the environment is intended for biologists interested in fungal molecular ecology and for fungal molecular ecologists interested in analytical tools beyond their particular expertise. We attempt to critically cover a wide variety of biochemical and molecular methods for fungal profiling, identification, and quantification, so that the reader can decide which are appropriate for his or her question. For reviews of fungal phylogenetics and fungal population genetics, see Berbee and Taylor (2001) and Carbone and Kohn (2004), respectively.

Morphologically based methods are the classic way of identifying species and assessing fungal diversity *in situ* (van der Heijden et al., 1998; Bever et al., 2001; Smith

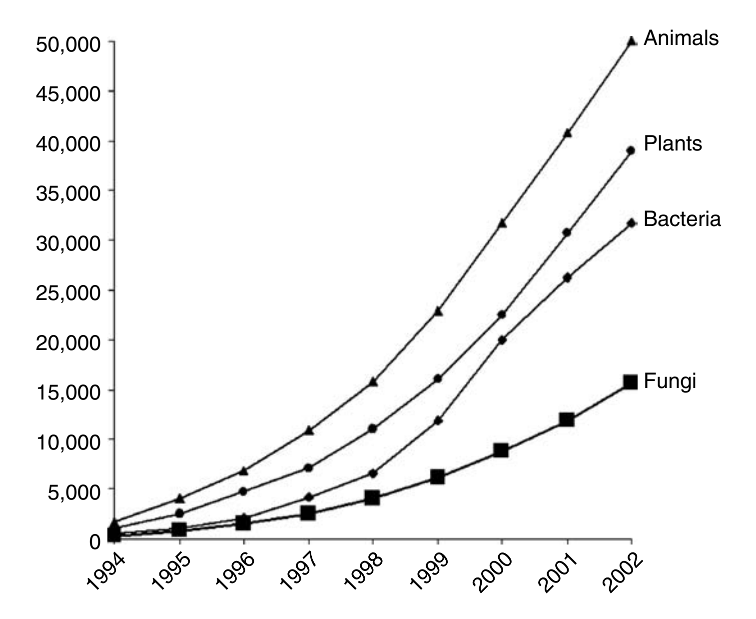


Figure 11.1 Cumulative taxonomic nodes (families, genera, and species) in GenBank on a yearly basis.

et al., 2002). These methods, although popular, are ultimately limited because the spore-bearing structures they rely upon are inconsistently produced or detectable and the correlation between reproductive structures and vegetative structures is sometimes poor (Clapp et al., 1995; Gardes and Bruns, 1996). Furthermore, the classical methods often overlook asexual, cryptic, and obligately biotrophic species, all of which are exceptionally common in fungi. Axenic isolation-dependent methods to assess diversity are highly selective; only ca. 17% of the 70,000 described species of fungi have been successfully cultured (Hawksworth, 1991). To overcome the limitations imposed by morphology and culturability, and to obtain a comprehensive view of fungal organismal biology and fungal communities, ecologists have been intensively using molecular methods since the 1990s; these methods have been applied successfully to vegetative structures, as well as reproductive structures, in many complex natural environments. The information gained can provide the basis for the statistical analysis of fungal communities to infer the processes that regulate the maintenance of diversity. However, because fungi (like bacteria or insects) are too diverse to count, exhaustive inventories of community richness remain impractical. Accordingly, community-level studies typically address how diversity or the abundance of selected species changes in relation to the abiotic, biotic, and temporal heterogeneity of the environment within a comparative framework.

DNA-based approaches have confirmed the extensive diversity of fungal communities and revealed previously undetected diversity (Redecker, 2000; Berch et al., 2002; Vandenkoornhuyse et al., 2002; Schadt et al., 2003). The discovery of genomic regions with DNA sequences conserved across entire clades and flanking variable regions led to the design of universal primers for the polymerase chain reaction (PCR). Five major strategies have since been developed to qualitatively and quantitatively analyze the composition of environmental samples containing multiple lineages, and they are consequently emphasized in this chapter:

1. PCR products can be separated electrophoretically to obtain whole-community genetic profiles and putative lineage assignments (e.g., via DGGE, RFLP, or T-RFLP; see Table 11.1).
2. PCR products can be sequenced and unknown taxa assigned to a phylogenetic clade.
3. Taxon-specific oligonucleotide probes can be designed and used in hybridization experiments against target molecules to assess diversity in environmental samples (e.g., oligonucleotide fingerprinting, microarrays).
4. PCR can be used to quantify the concentration of DNA to estimate the biomass of a target lineage via end-point or real-time automated techniques (i.e., competitive PCR, quantitative PCR).
5. Sequence-specific fluorescent probes can be used for direct absolute counts of the organisms in a target clade via FISH, or PCR can be combined with *in situ* hybridization within intact cells or tissues to attain the same goal.

This review provides an overview of the advantages as well as the technical limitations of both biochemical and DNA-based approaches used in fungal molecular ecology and discusses recent methodological developments (i.e., microarrays) not yet applied to fungi. For a glossary of terms and acronyms used in fungal molecular ecology and throughout this chapter, see Table 11.1.

11.2 BIOCHEMICAL METHODS FOR ENVIRONMENTAL MONITORING

11.2.1 Substrate Utilization Assays Using Microtitration Plates

Differences in physiological and metabolic activities between fungal species can be determined as patterns of substrate–source utilization (most often C sources) using 96-well microtitration plates originally developed for the identification of single bacterial strains. There are three main ways in which fungal characterizations have been carried out to assess biochemical and community diversity using substrate utilization profiles:

1. Single fungal strains have been used to inoculate a microtitration plate and each well has been scored for fungal growth after incubation (Wildman, 1995).
2. Soil fungal community-wide metabolic potential has been monitored by turbidimetry, which is correlated with biomass accumulation, in the presence of antibacterials (Buyer et al., 2001) and using commercially available fungal plates (SFN2 and SFP2, BIOLOG Inc.) (Classen et al., 2003).
3. Fungal activity has been estimated in the presence of antibacterials after inoculation with decomposing litter (Dobranic and Zak, 1999) or soil organic matter (Sobek and Zak, 2003) by adding a dye (dimethylthiazolyl–diphenyltetrazolium bromide (MTT)) that is reduced by fungi.

The fungal communities that develop in individual wells have not been characterized, but they probably comprise fast-growing fungi that can grow in liquid and on a single substrate (e.g., many yeasts). Thus, it is unlikely that substrate utilization assays will provide a comprehensive view of community diversity, either functional or phylogenetic. However, they will remain useful to identify culturable plant pathogens, clinically important fungi, and strains used by industry.

Table 11.1 Glossary of Terms and Acronyms Used in Fungal Molecular Ecology

Term	Definition
AFLP	Amplified fragment length polymorphism.
ARDRA	Amplified ribosomal DNA restriction analysis.
BIOLOG	A commercially available microtitration plate for substrate utilization assays.
Calibrator	A sample used as the basis for comparative results.
CGA	Community genome (micro)array; it is constructed using genomic DNA isolated from axenic strains.
Chimera	A sequence generated when an incompletely extended PCR product acts as a primer on a heterologous sequence.
Competitive PCR	End-point PCR quantification using coamplification of an internal standard.
DGGE	Denaturing gradient gel electrophoresis.
Exogenous control	DNA added to samples at a known concentration.
FAME	Fatty acid methyl ester.
FGA	Functional gene (micro)array; it contains DNA encoding enzymes involved in ecosystem processes (e.g., nitrogen cycling).
GC-FAME	Gas chromatography fatty acid methyl ester analysis.
Heteroduplex	A sequence formed by cross-hybridization of heterologous sequences.
Hot start	A method to avoid annealing during PCR until a threshold temperature is reached in order to minimize nonspecific annealing, usually by using a reversibly inactivated polymerase.
Immunocapture PCR	Template enrichment by binding antibody–cell complexes to magnetic beads; recovery from solution with a magnet, DNA isolation, and PCR.
Inhibition	PCR failure due to the presence of extraneous chemicals despite the presence of sufficient template.
Internal positive control	DNA used to distinguish true target negatives from PCR inhibition, or to normalize for differences in sample extraction.
ITS	Internal transcribed spacer of the ribosomal repeat.
Metagenomics	Archiving environmental DNA in genomic libraries (up to 100 kb) without cultivation or PCR.
Microarray	Orderly arrangement of large sets of DNA molecules of known sequence for hybridization experiments.
MPN	Most probable number, a statistical method for estimating abundance.
Multiplex PCR	PCR using more than one primer pair.
Nested PCR	Using PCR products as templates for a second PCR.
OFRG	Oligonucleotide fingerprinting of ribosomal genes.
Phylo-placement	Identification using phylogenetic methods of the least inclusive monophyletic clade to which a DNA sequence of unknown origin belongs.
PLFA	Phospholipid fatty acid analysis.
POA	Phylogenetic oligonucleotide (micro)array or "PhyloChip." Contains oligonucleotide probes derived primarily from ribosomal gene segments.

Table 11.1 Glossary of Terms and Acronyms Used in Fungal Molecular Ecology (Continued)

Term	Definition
Real time	Online quantification during logarithmic phase of PCR.
Reporter gene	A gene whose protein product is quantified or visualized.
RFLP	Restriction fragment length polymorphism.
Selectivity	Inclusion of all intended target templates (= universality).
Sensitivity	Minimum number of detectable templates.
Specificity	Exclusion of all unintended target templates.
SSCP	Single-stranded conformational polymorphism.
Standard	A sample of known concentration used to construct a standard curve.
TGGE	Temperature gradient gel electrophoresis.
T-RFLP	Terminal restriction fragment length polymorphism.
TSOP	Taxon-specific oligonucleotide probe.

11.2.2 Profiling Using Phospholipids (PLFAs or FAMEs)

Phospholipids are essential components of cell membranes, and they rapidly degrade after cell death via enzymatic action. In the lipid analysis method, phospholipids are extracted using various solvents and then fractionated into different categories. Phospholipid fatty acids (PLFAs) that are liberated from the phospholipid fraction are transformed to fatty acid methyl esters (FAMEs), which are identified and quantified using gas chromatography. This biochemical method has been used to monitor shifts in overall microbial community structure and in community subsets such as bacteria, actinomycetes, and fungi in soil (Zelles, 1999; Myers et al., 2001). Measurements of stable isotope (as ^{13}C) incorporation into PLFAs can also be used to detect changes in metabolic activities of organisms (Arao, 1999). However, relatively little information has been compiled so far on fatty acid composition of fungi (Graham et al., 1995; Madan et al., 2002), and it is unlikely that phospholipids will provide detailed pictures of fungal community structure in the future. Because many fatty acids are common to different species of fungi, they have limited value for identification of the organisms from which they derive, especially when complex fungal communities are analyzed (Olsson, 1999; Zelles, 1999). Another important limitation is that phospholipid concentration can change with the physiological state of the organism in response to environmental factors (Olsson, 1999; Zelles, 1999). In summary, this technique appears useful as a complementary approach for gross characterization of communities (Hedlund, 2002; Olsson et al., 2003) or as a living biomass estimator for subsets of fungi in laboratory experiments (Olsson et al., 1998; Olsson, 1999; Wallander et al., 2001).

11.3 DNA-BASED METHODS FOR ANALYZING COMMUNITY DIVERSITY

In the 1980s, emphasis was placed on restriction fragment length polymorphism (RFLP) patterns of ribosomal genes to characterize fungal strains using DNA hybridization. Axenic isolates were often used because large amounts of pure DNA were required to perform the analysis. The discipline of molecular ecology emerged in the 1990s with the develop-

ment of PCR technology, which considerably increased the sensitivity and specificity of all molecular tools (Mullis and Faloona, 1987). Within a few years, progress was made in the development of fungal primers (White et al., 1990) and application of PCR to environmental samples for community analysis without the need for cultivation (Gardes et al., 1991; Henrion et al., 1992; Simon et al., 1992; Gardes and Bruns, 1993). In principle, these techniques can be applied to any gene. However, ribosomal DNA molecules have been the most popular in fungal ecology for three main reasons:

1. They are present and typically in multiple identical copies within all organisms.
2. They comprise highly conserved sequence domains that have allowed for the design of fungal-specific primers (White et al., 1990; Gardes and Bruns, 1993).
3. Their sequence variation spans various hierarchical levels of phylogenetic diversity, from species to kingdoms.

More than a decade later, numerous complementary techniques are now available to assess the diversity of fungal communities. We will examine the principles, advantages, and limitations of PCR-based methods and illustrate their applications in community studies with a few selected examples. A summary is provided in Table 11.2.

11.3.1 Rapid Profiling Techniques

Fingerprinting techniques have been developed to profile mixed fungal populations in a quick, reliable, and cost-efficient way. They all rely on extraction of environmental DNA, amplification of targeted sequences, and electrophoresis of PCR products in an agarose or polyacrylamide gel to generate genetic fingerprints. Differential migration of the amplified fragments depends on their length, their sequence and/or their conformation. Unless they are automated, rapid profiling methods produce results that can vary from one experiment to another and do not allow for the development of high-resolution databases.

11.3.1.1 Profiling Based on Size Differences of PCR Products after Restriction Digestion: PCR-RFLP and T-RFLP

The earliest molecular attempts to analyze fungal communities relied heavily upon PCR amplification of the internal transcribed region of the nuclear ribosomal unit (internal transcribed spacer, ITS), followed by digestion of the amplified product using a few tetrameric restriction enzymes, and finally electrophoretic separation of the fragments. The patterns obtained from different species are polymorphic due to mutations in the restriction sites and to base insertions or deletions (indels) within the amplified fragments. In fungi, the ITS consists of two noncoding spacers, ITS1 and ITS2, separated by the highly conserved 5.8S rRNA gene. It is typically amplified using either universal eukaryotic or fungal-specific primers targeted to conserved regions in the 3′ end of the 18S rRNA gene (small subunit, SSU) and the 5′ end of the 28S rRNA gene (large subunit, LSU). The ITS region has been extensively used in fungal ecology because a low level of intraspecific variation and a high level of interspecific variation were empirically observed. Thus, for example, most studies of ectomycorrhizal fungal communities have involved the following steps: (1) PCR amplification of the fungal symbiont from individual root tips, (2) digestion of the amplified DNA product, (3) separation of the restriction fragments by electrophoresis, and (4) comparisons of patterns with restriction fragment length polymorphism databases from sporocarps (for a review, see Horton and Bruns, 2001). This relatively simple approach is also commonly used as an intermediate screening step prior to sequencing PCR clones obtained from soil DNA samples (Viaud et al., 2000; Chen and Cairney, 2002).

Table 11.2 Advantages and Limitations of PCR-Based Fingerprinting Techniques

Method	Acronym	Advantages and Limitations
Denaturing gradient gel electrophoresis	DGGE	A rapid profiling technique for detecting community changes; does not provide phylogenetic information directly; a low-resolution alternative or a screening tool for sequence-based methods
Temperature gradient gel electrophoresis	TGGE	Idem as DGGE
Single-stranded conformational polymorphism	SSCP	Idem as DGGE; possibility of automation and standardization using fluorescence-based technologies
Restriction fragment length polymorphism	RFLP	A rapid profiling technique extensively applied in fungal ecology using ribosomal genes and spacers; a low-resolution alternative or a screening tool for sequence-based methods
Terminal RFLP	T-RFLP	A modification of RFLP using fluorescence-based technologies with higher throughput, resolution, and reproducibility; a lower-resolution alternative or a screening tool for sequence-based methods
Sequencing and phylogenetic affiliation	—	The state of the art in community analysis; limited by the pitfalls of PCR and the phylogenetic saturation of DNA databases
Oligonucleotide fingerprinting	TSOP	Hybridization-based identification that permits taxonomic discrimination using taxon-specific oligonucleotide probes (TSOPs); reliable and cost-effective, but its optimization is labor-intensive
Microarrays for detection	POA FGA CGA	Potentially the most powerful hybridization-based identification system; limited by (1) the design and testing of probes, (2) the sensitivity and specificity of the hybridization step, and (3) the processing of results

The accuracy of RFLPs is highest when they are automated and run on polyacrylamide gels using PCR products generated with fluorescently labeled nucleotides (Peter et al., 2001; Kennedy et al., 2003). A recent modification to increase the resolution of the RFLP technique for multitemplate sample screening involves the use of fluorescently tagged oligonucleotide primers for PCR amplification and subsequent automated electrophoresis of the fragments generated. Only the end-labeled fragments are detected, which reduces the complexity of the profile. This technique, referred to as T-RFLP (terminal restriction fragment length polymorphism) is a rapid and sensitive approach that can be used to assess the similarities between communities (Liu et al., 1997). However, the richness and evenness of a community are only qualitatively estimated, and they depend on the restriction enzyme used to derive the profile. In order to select optimal restriction enzymes, optimization of the screening step is possible using computer-simulated RFLP analysis of DNA sequence database accessions (Moyer et al., 1996; Marsh et al., 2000; Lord et al., 2002). A basic limitation of T-RFLP is that all bands are counted equivalently, and consequently the phylogenetic disparity among DNA sequences is unknown. In addi-

tion, its interpretation is problematic for organisms that contain different rDNA repeats within individuals (i.e., arbuscular mycorrhizal fungi; Vandenkoornhuyse et al., 2003). Another potential problem is the formation of pseudoterminal restriction fragments (Egert and Friedrich, 2003).

To gain insight into the phylogenetic composition of the community, terminal restriction fragments can be recovered from gels, cloned, and sequenced (Nagashima et al., 2003). Alternatively, the construction of single-strain profile databases directly from specimens, or indirectly from DNA sequences retrieved from databases if their amplification primers are known, is an efficient approach. In summary, considering the high-throughput capacity and sensitivity of T-RFLP (Moeseneder et al., 1999), it is likely that it will become one of the most useful rapid profiling methods for the study of complex fungal communities in the near future. It has been used already to detect and identify fungal mycelia in forest soils and decaying plant leaves (Buchan et al., 2002; Dickie et al., 2002; Klamer et al., 2002; Nikolcheva et al., 2003), and for the identification of ectomycorrhizal fungi from mycorrhizae and sporocarps (Zhou and Hogetsu, 2002; Nara et al., 2003).

11.3.1.2 Profiling Based on Differences in Melting Temperatures: DGGE and TGGE

In denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE), DNA fragments of the same size are separated through a linear gradient of increasing denaturant (chemical or heat, respectively). In such environments, double-stranded DNA molecules partially unwind and undergo both a conformational and mobility change. For two fragments with the same sequence length, the melting temperature (Tm) of the DNA molecule is determined by the proportion and position of G + C bases. Both techniques are considered interchangeable (Heuer et al., 1997), and they are both capable of separating nearly identical sequences. Community patterns can subsequently be interpreted either by comparison with reference patterns or phylogenetically when individual bands in the profile are assigned to taxonomic units by hybridization or sequencing. A significant amount of work must be undertaken to optimize the separation of the PCR products before screening, and relatively long GC-tailed primers must be used to stabilize the transitional molecule. In addition, a single band in community profiles may be composed of several unrelated sequences (Schmalenberger and Tebbe, 2003). Such comigrating fragments are relatively common when environmental samples are analyzed. Resolution is also affected by the gel staining procedure and the electrophoretic conditions. To improve resolution, the community can be divided into smaller phylogenetic groups using taxon-specific primers that are then individually analyzed.

DGGE and the related technique TGGE have been increasingly popular tools to qualitatively describe bacterial communities since the first report of DGGE profiles of bacterial mats and biofilms (Muyzer et al., 1993). They offer a relatively good compromise between the number of samples processed and the information that can be obtained (Muyzer and Smalla, 1998). One of the first applications of DGGE to fungal communities was a study of pathogens in plant roots (Kowalchuk et al., 1997). In this study, a nested PCR approach was used to specifically recover fungal 18S rDNA sequences from infected roots. The amplified products were subjected to DGGE analysis, and the major bands excised, reamplified, cloned, sequenced, and included in a phylogenetic analysis. The sequence data revealed fungal diversity not detected in previous isolation-based surveys, and the presence of several taxa not related to the existing fungal 18S rDNA sequences in the databases. In a related study on the same plant and habitat, the DGGE strategy proved useful in comparing communities of arbuscular mycorrhizal fungi, as well as

comparing the arbuscular mycorrhizal fungi detected in spore banks vs. living plant roots (Kowalchuk et al., 2002). A similar procedure was developed by Smit et al. (1999) using TGGE to compare fungal diversity in a wheat rhizosphere vs. bulk soil in a microcosm experiment. These rapid profiling methods have also been used to analyze fungal communities in other complex habitats, such as historical church window glass (Schabereiter-Gurtner et al., 2001), tree stumps (Vainio and Hantula, 2000), and grassland (Brodie et al., 2003) and maize rhizospheres (Gomes Newton et al., 2003).

11.3.1.3 Profiling Based on Differences in Conformation: SSCP

In single-stranded conformational polymorphism (SSCP) analysis, PCR products of the same length but with different sequences are converted into profiles composed of multiple bands that migrate differently under nondenaturing conditions because of their conformation. SSCP is based on the principle that single-stranded DNA adopts a tertiary structure that is sequence specific (even to single-nucleotide polymorphisms). However, depending on the resolution of the gel, a single band may contain more than one sequence (Schmalenberger and Tebbe, 2003). Similarly to DDGE and TGGE, it is a technique that was originally developed to detect point mutations and allelic variants in human genes using electrophoretic gels (Orita et al., 1989; Hayashi, 1991). However, this technique does not require the construction of gradient gels, and its sensitivity and reproducibility have been significantly improved using automated fluorescent electrophoresis (Lee et al., 1996; Zumstein et al., 2000), which notably facilitates comparisons. PCR combined with SSCP analysis has also been applied to microbial communities as an alternative fingerprinting approach to DGGE or TGGE (Lee et al., 1996; Schmalenberger and Tebbe, 2003). Despite its potential as a relatively simple and effective method for the detection of minor sequence changes in PCR-amplified products, this method has been rarely applied to fungal communities. Earlier attempts were made to use PCR-SSCP for the identification of fungal species from roots, axenic isolates, or spores using the ribosomal genes and spacers (e.g., Simon et al., 1993; Kumeda and Asao, 1996; Moricca and Ragazzi, 1998; Kjøller and Rosendhal, 2000). To our knowledge, only one preliminary study has applied SSCP to changes in fungal communities in response to environmental factors (Lowell and Klein, 2001).

11.3.2 Sequencing and Phylogenetic Affiliation

11.3.2.1 Identification through Phylogenetic Placement

The PCR, clone, and sequence approach to studying microbial community structures can be thought of as a gold standard. It is, or should be, used to validate screening techniques (e.g., DGGE, T-RFLP, SSCP). However, bacterial and archaeal 16S community studies have clearly highlighted the biases and artifacts introduced by this popular approach (Qiu et al., 2001; Speksnijder et al., 2001), as well as its practical limitations (Dunbar et al., 2002). Biases are due to over- and underrepresentation of phylotypes, depending on amplification and cloning conditions used for multitemplate samples. Artifacts are extensive and varied, and they will be discussed in detail in Section 11.3.3.1.

The practical limitations of the PCR, clone, and sequence approach in comparative bacterial community analysis have been highlighted by Dunbar et al. (2002), both by reanalyzing empirical data and through modeling. While species richness is likely to be reproducibly estimated with the typical number of clones analyzed per soil sample, species composition is not, and it would require orders-of-magnitude-larger samples than those typically used. A recently developed alternative to commonly used, but highly sensitive

diversity indices, lineage-per-time plots are a robust method for phylotype frequency estimation (Martin, 2002). The use of this and other analytical tools for molecular biodiversity data has been reviewed recently (Hughes et al., 2001; Curtis et al., 2002; Bohannan and Hughes, 2003).

The molecular analysis of ectomycorrhizas, typically screened by RFLP and followed by direct sequencing, has been relatively straightforward to apply to large numbers of individual root samples because sufficiently pure single-strain fungal DNA can be obtained from roots without cloning (Horton and Bruns, 2001). High-throughput sequencing using capillary arrays has greatly accelerated this trend (>60,000 roots were analyzed by Tedersoo et al., 2003). Identification of ectomycorrhizal fungi in soil outside roots has required cloning (Landeweert et al., 2003) or T-RFLP (Dickie et al., 2002). In contrast, there is yet no widely used optimal molecular method to assess diversity in arbuscular mycorrhizas, particularly in field roots or soil. This is because

1. The fungi colonize individual roots less extensively than ectomycorrhizal fungi, so that target template molecules are less abundant relative to PCR inhibitors.
2. Fungal rDNA is highly diverse and under relaxed concerted evolution, so that primer design is problematic (Schüßler et al., 2001), cloning is usually necessary, and sequence interpretation is troublesome.
3. No genes of phylogenetic utility other than nuclear rDNA have yet been widely sequenced and made available.
4. The fungi contain endosymbiotic ascomycetes and bacteria.

Here we briefly describe 4 representative methods (of over 20 published to date) selected based on their popularity or uniqueness. Helgason et al. (1998) directly amplified, cloned, and sequenced approximately 500 base pair (bp) of the 18S gene with one primer set. Simon et al. (1993) used nested PCR with a eukaryotic primer set, and then amplified approximately 150 to 400 bp with three lineage-specific primer sets. Clapp et al. (1995) used a selective enrichment PCR approach targeting approximately 150 bp of 18S rDNA with four steps: (1) PCR with a universal primer set was done on root and leaf samples, using a biotinylated base for leaf samples; (2) leaf and root products were combined, denatured, and reannealed; (3) plant DNA was subtracted using streptavidin; and (4) PCR with three lineage-specific primer sets was done. Redecker (2000) used a nested PCR approach targeting approximately 1 kb of rDNA (3′ 18S + ITS1) with a eukaryotic primer set, and then with five lineage-specific primer sets. In optimal conditions, fungi can be stained and visualized in the root tissue after DNA extraction. The last approach was the only one to target all known glomalean lineages to date. Thus, although popular, single-primer studies of arbuscular mycorrhizal communities must be regarded with caution. Nonetheless, all approaches can amplify nonglomalean fungi, and all lineage-specific primer sets can cross-amplify glomalean lineages so that sequencing exemplars were always necessary to validate putative phylo-placements.

11.3.2.2 Oligonucleotide Fingerprinting and Microarrays

Oligonucleotide fingerprinting and microarrays are hybridization tools that can be used as genomic identification systems through the analysis of small DNA segments. In these systems, diversity among DNA sequences is exploited to identify organisms.

In conventional oligonucleotide fingerprinting, an array is constructed by applying spots of PCR-amplified DNA onto a nylon membrane, which is then subjected to a series of hybridization experiments (often referred to as dot-blots), each using a single DNA

taxon-specific oligonucleotide probe (TSOP). Fingerprinting works by sorting arrayed DNAs into taxonomic clusters based on their hybridization patterns. When fully developed and tested, this identification system provides a reliable and cost-effective method for high-throughput species identification in multitemplate environmental samples. However, this tool has been slow to be applied in molecular ecology, which is explained partly by the time and labor required for the development of an operational identification system. The literature offers a few examples of the successful application of TSOPs for the analysis of fungal communities using conventional dot-blot experiments (e.g., Bruns and Gardes, 1993; Valinsky et al., 2002).

DNA microarrays (or DNA chips) were originally designed for large-scale sequencing and genetic analyses (Southern et al., 1992; Guo et al., 1994), and they represent a significant advance in hybridization technology. They are used extensively in functional genomics (for fungi, see Cavalieri et al., 2000; Nowrousian et al., 2003). Microarrays also hold much promise as tools in environmental studies because of their exceptional high-throughput capacity. Thus, phylogenetic oligonucleotide microarrays (POAs) will allow for the simultaneous application of hundreds of thousands of TSOPs in a single hybridization experiment. POA is a reversed hybridization system; matrix-immobilized oligonucleotide probes are used to capture labeled target molecules (e.g., amplified DNA segments, rRNAs). In addition to qualitative analysis of communities (Loy et al., 2002; Wilson et al., 2002), phylogenetic oligonucleotide microarrays (or PhyloChips) have the potential to be sensitive enough for quantitative detection of intact rRNAs from environmental samples (Small et al., 2001). However, to realize the full potential of POAs for the analysis of fungal communities, significant advancements will need to be made in (1) the design and validation of TSOPs (Wilson et al., 2002); (2) the sensitivity and specificity of the hybridization procedure and technology, particularly for soil samples (Small et al., 2001; Urakawa et al., 2003); and (3) the processing of hybridization results (Townsend, 2003). In addition, two kinds of microarrays can be used to monitor the activities of organisms in microbial communities (for a review, see Zhou and Thompson, 2002). First, functional gene arrays (FGAs) containing genes involved in ecosystem processes (e.g., nitrogen cycling) can be used to reveal the physiological and metabolic activities of living organisms in environmental samples (Wu et al., 2001). Second, community genome arrays (CGAs) can be constructed using whole genomic DNA from pure strains to examine their activities in the environment. Both of these remain to be tested in fungal ecology.

11.3.3 Pitfalls of PCR-Based Fingerprinting Methods

Two main sources of experimental error have been recognized: (1) PCR biases and artifacts, and (2) interpretation of the data (Wintzingerode et al., 1997; Martin, 2002; Bruns and Shefferson, 2004). Sample processing, cell lysis, and nucleic acid extraction are significant sources of variation for all subsequent analyses using a PCR approach (Stach et al., 2001), but surprisingly, they appear to be very rarely optimized.

11.3.3.1 PCR Biases and Artifacts

PCR amplification requires special attention to several problems intrinsic to experiments with multitemplate samples. With some primer–template combinations, some phylotypes can be disproportionately amplified due to preferential priming or differences in elongation rates among amplicons (Wintzingerode et al., 1997). A different bias can occur when a reaction undergoes too many cycles because there is a tendency for the different amplicons to reach equimolarity because reannealing of frequent PCR products progressively inhibits primer–template annealing (Suzuki and Giovannoni, 1996). If quantitative inferences of

relative phylotypes are intended, adequate controls have to be included for each primer–template (Lueders and Friedrich, 2003).

Another set of potential PCR-generated artifacts (i.e., heteroduplexes, chimeras, and mutagenesis) have received much attention in the context of PCR, cloning, and sequencing approaches (Qiu et al., 2001; Speksnijder et al., 2001), but artifacts are also sources of spurious bands (or peaks) in rapid profiling methods. For instance, artifacts specific to T-RFLP have been recently reported (Egert and Friedrich, 2003). PCR artifacts may lead to the commonly observed "bushes" of closely related cloned sequences. Detecting such aberrant microvariation is a problematic and largely unresolved concern. *Heteroduplexes* are heterologous hybrids formed from different gene templates; some heteroduplexes can separate from homoduplexes during electrophoresis (due to conformational differences), thereby producing additional bands or peaks, which may be removed by gel purification. The occurrence of heteroduplexes increases with increasing similarity between sequences; this may happen even within individuals for multicopy genes. When heteroduplexes are cloned, they can be converted into a single hybrid sequence that is a mosaic of the two parent heterologs (because the insert is not methylated, excision-repair enzymes cannot distinguish parent DNA strands and independently use either strand as a template for each base). In any multitemplate PCR, heteroduplexes can be as abundant as homoduplexes (Thompson et al., 2002) and can make up to 10% of clones (Lowell and Klein, 2000). Heteroduplexes can be resolved before genomic library construction by digestion with a single-strand cleaving endonuclease (Lowell and Klein, 2000), or they can be minimized by nested PCR, increasing primer concentration, and using a low number of cycles for reamplification (Thompson et al., 2002). *Chimeras* are sequences generated when an incompletely extended PCR product acts as a primer on a heterologous sequence. For SSU rDNA, there are programs that can detect potentially aberrant variation (e.g., Chimera Check). In a recent study, 27 of 29 environmental fungal phylotypes recovered were thus assessed to be potentially chimerical (Jumpponen, 2003). *PCR mutagenesis* can be due to base misincorporation, or deletions when the template has secondary structure, and can be minimized by the use of DNA polymerase mixtures that contain a proofreading enzyme. In general, harvesting PCR products as early as possible during the exponential phase of the reaction is widely regarded as the most efficient way of minimizing all artifacts. While DNA sequencing is as sensitive to chimeras as rapid DNA profiling methods, the latter are more sensitive to heteroduplexes and mutagenesis (Qiu et al., 2001). Clean sequences cannot be obtained if a clone results from a heteroduplex, and single-base mutations may have little or no effect on overall phylogenetic topology. However, heteroduplexes and mutagenesis can have potentially large impacts on nonsequencing methods (i.e., RFLP, T-RFLP, DGGE, TGGE, SSCP).

11.3.3.2 Data Interpretation Pitfalls

It must be noted that DNA-based ecological methods still rely heavily on single-locus analysis of multicopy noncoding gene regions. However, multilocus genotyping provides much more precise molecular identification of species. For a review of how DNA sequence data alone can be used to define and recognize fungal species through the use of multigene phylogenies (i.e., the phylogenetic species concept), see Taylor et al. (2000). The use of single-copy genes avoids paralogous comparisons, and sequences from protein-coding genes are significantly less ambiguous to align than noncoding DNA. The realization of both of these loci's potential for fungal identification from environmental samples awaits further growth of their representation in the public database. However, the PCR amplification single-copy genes and protein-coding genes from the environment

remain problematic, largely due to insufficient template concentrations or difficulties in universal primer design. Some progress has been made in the identification and use in environmental samples, such as mycorrhizas, of rapidly evolving repetitive DNA sequences (i.e., microsatellites) from key fungal species (Kretzer et al., 2003). These allow for high-resolution assessments of intraspecific structure, including the identification of fungal individuals (genets).

DGGE, TGGE, SSCP, and T-RFLP are adequate methods for rapidly comparing whole fungal communities, but they often are unable to provide reliable information describing community structure (richness, evenness, and phylo-divergence) without any DNA sequencing. However, because the size of the products from profiling techniques must be short (maximum length of about 500 bp for DGGE, TGGE, and SSCP), only a broad phylogenetic affiliation can usually be obtained via DNA sequencing.

The generation of reliable DNA sequence data that can be used for the identification of taxa from the environment is a major but often unrecognized contribution of molecular systematics studies and, increasingly, molecular ecology studies. Fungal molecular identification is unrivaled when it comes to situations where only vegetative structures are available, which is the case in the vast majority of situations for the vast majority of fungi. No other approach offers greater discriminatory power (i.e., the ability to determine that $x = y \neq z$). However, obtaining a phylogenetic placement (e.g., species, genus, or family) is only possible when similar sequences from closely related and identified fungi are available for comparison. In other words, while molecular identification is a gold standard, the phylo-placement used to achieve it is only as good as the level of saturation in molecular diversity of the database for the target group. For instance, a recent study based on environmental fungal DNA sequence surveys at a Scottish site showed major phylo-placement limitations; only approximately 10% of sequences could be confidently assigned to a genus (Anderson et al., 2003). Conversely, an easily overlooked cause of organismal richness underestimation is that *primer selectivity* (universality) and *primer specificity* are rarely strict. It is known that for glomalean fungi this is a major concern (Schüßler et al., 2001; Morton and Redecker, 2001) and for basidiomycetes it is a relatively minor concern (e.g., sebacinoids, tulasnelloids).

The public nucleotide and protein *sequence database* is one of the pillars of molecular biology, molecular evolution, and molecular ecology. However, the quality control features of the database are surprisingly shortsighted and highly limited (e.g., the staff-curated exemplar sequence database RefSeq contains whole-chromosome sequences from yeast as its sole fungal sequences), and updates or changes to existing GenBank sequence data from another submitter can be submitted only as new sequences, and they are eroding the database's accrual of value. Currently, sequence data are annotated by their authors and subsequently only reannotated by the same authors. While attention has been recently drawn to sequence errors (Blaxter and Floyd, 2003; Harris, 2003), peer-reviewed reports of annotation errors are being published as well. For instance, in three well-represented groups of fungi, up to 20% of sequence accessions may have erroneous lineage designations in GenBank (Bridge et al., 2003). This is an unfortunate state of affairs and one that can be solved with a dose of long-term perspective. Since the origin of public zoological and botanical specimen collections, an open system of incremental annotation has evolved. This was needed as new specimens allowed for reevaluations of older specimens and the original depositors were gradually becoming unavailable, just as will happen with today's molecular biologists, evolutionists, and ecologists. As DNA-bar-coding efforts get under way to catalog the world's biodiversity (Blaxter, 2003), the time has come for the public sequence database to incorporate an open-access cumulative annotation system that can accommodate an exponential phase in sequence submission likely to last for at least another

decade. Leaving quality control of a permanent tool to impermanent users was acceptable for the first decade, but it is no longer.

Fortunately, relying on phylo-placement instead of merely percent identity for molecular identification purposes can help to detect and minimize the effects of poor sequence quality and erroneous annotation (for some examples, see Bruns and Shefferson, 2004). However, another critical concern is that currently DNA sequence databases only contain representatives of a small and often arbitrary subset of the phylogenetic diversity in nature (and usually a single gene per taxon). Undoubtedly, there is a need for better sequence alignment methodologies that can achieve positional homology of sequences to accurately reconstruct phylogenetic associations among the numerous and widely divergent taxa that have been haphazardly targeted for sequencing so far. But ultimately, the main determinant of whether positional homology can be achieved during sequence alignment is the taxon richness of the alignment. In an rDNA fungal survey of the roots of one plant population, only 7 of 49 phylotypes were highly similar to available sequences (>99% identity), and 5 clades could not be assigned to a phylum (Vandenkoornhuyse et al., 2002). When closely related sequences are available, alignment and phylogenetic reconstruction are essentially routine tasks. Otherwise, long-branch attraction and other phylogenetic artifacts become major obstacles. In light of this, it is surprising that very few fungal specimen collections at public herbaria and museums are systematically brought online by concerted sequencing efforts. The Deep Hypha network is one limited attempt to remedy this situation (ocid.nacse.org/research/deephyphae).

11.4 DNA QUANTIFICATION, SPECIES DETECTION, AND BIOMASS ESTIMATION

11.4.1 Quantitative PCR

The quantitative PCR methods that have been applied to fungi fall into three main categories: end point using an internal standard, semiquantitative, and automated real-time quantification. *Competitive* PCR is an end-point method that relies on coamplification of a target template with an internal standard of comparable amplification efficiency (Siebert and Larrick, 1992). The amount of target DNA in a sample can be quantified by titrating unknown amounts of target DNA against a dilution series of the competitor internal standard. The internal standard can be

1. Homologous — constructed by insertion, deletion, or mutagenesis directed at a restriction site (e.g., Guidot et al., 2002, 2003; Mauchline et al., 2002) of the target DNA while avoiding heteroduplex formation, or
2. Heterologous — DNA screened and isolated from a distant organism (e.g., Edwards et al., 1997) or DNA modified to contain the priming sites of the target DNA by the use of chimerical primers (e.g., Baek and Kenerley, 1998).

Alternatively, one target may be used as competitor for another target to obtain relative amounts of two different fungi (e.g., Oba et al., 2002). Because the internal standard and target share primer sites, competition for amplification occurs and the end-point ratio of the two PCR products' concentration can be used to calculate the initial target concentration. However, absolute quantification is not obtained, because achieving identical amplification efficiencies of the target and competitor is not possible in practice, especially during late PCR cycles when primer concentration, nucleotide concentration, and polymerase activity become limiting. For each sample DNA extract, a competitive PCR assay

will consist of a dilution series with known amounts of competitor DNA and a constant concentration of target DNA. Conversely, a dilution to extinction series may be produced before PCR and end-point data gathered as only positives/negatives (a most probable number assay; e.g., Lozupone and Klein, 2002). This is a semiquantitative method without the use of any standards. Competitive PCR methods can be as reproducible and nearly as accurate as real-time quantification (Desjardin et al., 1998). They may involve lower material costs, but higher labor costs, and the numerous manipulations may increase cross-contamination and sample variation.

Automated *real-time* PCR uses a fluorogenic probe, and the amount of fluorescence detected is proportional to the amount of accumulated PCR product (preferably 50 to 150 bp long). The amount of target DNA is measured in each cycle of amplification; thus, samples are quantified during the exponential phase leading to the most accurate results. The critical threshold cycle value (Ct) refers to the PCR cycle number where detectable (i.e., above noise) amplification product is first measured. The Ct value is inversely proportional to initial DNA template concentration. The amplification efficiency for a primer set is determined from the slopes of plots of Ct values vs. serially diluted DNA extracts, and ideally it spans several orders of magnitude. Real-time PCR has been extensively applied in biomedical molecular diagnostics for highly sensitive and rapid microbial pathogen detection. For field detection, reaction times under 10 min have been reported for the bacterium *Listeria* (Belgrader et al., 1999). The gene expression applications of real-time PCR have been reviewed by Giulietti et al. (2001) and its biomedical applications by Klein (2002).

For absolute quantitation via real-time PCR, one needs known quantities of PCR standards determined by an independent means. The amount of target DNA in a sample is interpolated from a standard curve run simultaneously with the unknown sample. For instance, Heuser and Zimmer (2002) quantified rDNA of three ascomycetes on *Quercus* leaves with the plasmid pCR*myr*S (10^5 copies) added as a standard directly before DNA isolation to each leaf. This is an ideal, but unusual approach; most quantitative PCR studies of environmental samples rely on a standardized DNA extraction protocol and addition of a standard or parallel amplification of standards at the PCR stage (Cullen et al., 2002; Cruz-Perez et al., 2001). Some studies have relied on relative quantitation; target DNA quantity is expressed relative to a control, or calibrator, without the need for a standard curve. This method relies on the amplification efficiencies of the target and calibrator being approximately equal. The comparative Ct method uses a formula for the amount of target, normalized to an internal reference and relative to a calibrator molecule derived from the PCR formula: $X_n = X_o (1 + E_x)^n$, where X_n = number of molecules at cycle n, X_o = initial number of molecules, and E_x = efficiency of target amplification (Roe et al., 2001; Haugland et al., 2002).

The oldest and most widely used real-time detection system, TaqMan (Applied Biosystems Inc.), uses two primers and a dual-labeled fluorogenic probe. In the intact fluorogenic probe, one dye quenches the other by fluorescence resonance energy transfer. The 5′ exonuclease activity of *Taq* polymerase cleaves the internally hybridizing probe during PCR, which leads to separation of the dyes in solution, reduced quenching, and increased fluorescence. Other detection systems also use intermolecular dyes in a separate probe (Loeffler et al., 2000) or more economical dyes that intercalate nonspecifically with double-stranded DNA (Schnerr et al., 2001; Heuser and Zimmer, 2002; Nakabachi et al., 2003; Alkan et al., 2004). If nonspecific dyes are used, primer dimers can be excluded by melting point temperature analysis (Schnerr et al., 2001). A highly specific (even to single-nucleotide polymorphisms) and kinetically efficient unimolecular approach is Scorpion-PCR, where the dyes, probe, and primer are designed as a single molecule (Bates and

Taylor, 2001; Schena et al., 2002). The available instrumentation and chemistries for the different real-time detection systems have been described by Giulietti et al. (2001).

Quantification of filamentous fungi is problematic because these fungi are not composed of discrete units of constant size and genetic content (e.g., spores often have a different number of genome copies than other cells). Most fungal studies quantify rDNA, but it is not straightforward to convert rDNA concentration to genome copies. And for any gene, it is also not straightforward to convert genome copies to biomass. This is a concern not just with poorly characterized organisms. What portions of quantified DNA correspond to decaying biomass or debris generated during DNA extraction, resistant propagules, spores, active hyphae, sporocarps, infective or colonizing units, etc.? Recovery from DNA extraction is typically assumed to be 100%, but this is a rarely tested and unlikely assumption, because in addition to adhesion to plastic and glass surfaces, thorough nucleic acid purifications (with consequently high DNA loss) are typically necessary to remove inhibitors from environmental samples such as soil. In fact, even fungal DNA obtained from pure cultures may contain PCR inhibitors that are as potent as those found in environmental samples (Cruz-Perez et al., 2001). Inhibition can lead to false negatives, but these can be avoided in real-time PCR by using an internal positive control (IPC) DNA. The IPC DNA is added to the reaction tubes with its specific primers and fluorescent probe. In the resulting multiplex reaction, there is no competition for primer binding sites. If PCR inhibitors affect the target DNA, they will also affect the IPC DNA. Because the IPC DNA always produces the same Ct value, the presence of inhibitors can be detected by a shift in the IPC Ct value. For either competitive or real-time PCR, as long as there is a reference DNA with primers and probes being coamplified in the samples, inhibition should be detectable. Last, but not least, it is essential that real-time PCR approached be validated by an independent and well-understood method during the evaluation process, as well as by replication. For absolute quantification in particular, the availability of accurate standards is critical. Undoubtedly, validation and standardization will be greater challenges for some fungal systems (e.g., arbuscular mycorrhizal fungi; Alkan et al., 2004) than others (e.g., human mycoses). Nonetheless, applying multiplex real-time PCR (up to 12 targets per reaction is the current limit) would revolutionize fungal community analysis in many systems, especially when coupled with microarray technologies that are the ultimate in high-throughput multiplexing, but currently require relatively large amounts of template.

11.4.2 Visualization to Estimate Biomass

Fluorescence *in situ* hybridization (FISH) is the method of choice for cultivation-free, reliable, rapid microbe visualization and quantification in environmental and clinical samples. It relies on cell fixation, hybridization with 15-25-mer fluorescently labeled oligonucleotide probes (typically targeting SSU rDNA), removal of unhybridized oligonucleotides, and finally visualization and quantification by epifluorescence microscopy, flow cytometry, or confocal laser scanning microscopy, all of which require extensive optimization. There are specific protocols that modify FISH in order to enhance detection and quantification in complex samples (with soil still a major exception due largely to excessive background fluorescence), allow for analysis of activity or specific functions, and allow sorting into phylogenetic groups (reviewed by Wagner et al., 2003). The online database of FISH probes (probeBase) currently contains only four fungal probes (all specific to *Candida* spp.), which reflects the lack of use of FISH in fungi. A recent exception is a study of spatial microheterogeneity of the black yeast *Aureobasidium pullulans* as both spores and hyphae on leaf surfaces (Andrews et al., 2002).

Bioreporters are bioengineering constructs that fuse an environmentally or metabolically responsive promoter to a *reporter gene* (e.g., green fluorescent protein (GFP),

luciferases, ice nucleation protein, LacZ, Gus) (reviewed by Lindow, 1995; Leveau and Lindow, 2002). A change in the environmental or metabolic stimulus to which the promoter responds produces a change in the transcriptional activity of the promoter. This leads to a change in the abundance of the reporter protein that can be measured by an appropriate microscopy method (e.g., epifluorescence and confocal laser scanning microscopy in the case of GFP). The target microbe must be cultured, transformed, shown to not differ from the wild type, and then reintroduced to its environment (as a marked strain). In addition, the substrate of the target microbe, and the microbe itself, must not interfere with visualization (e.g., quenching, autofluorescence). Despite their popularity in prokaryote biology to monitor responses to nutrients, metals, temperature, etc., as well as growth rate or deficiencies in essential resources, bioreporters have been only exceptionally used in fungal ecology. Olivain and Alabouvette (1997) used a strain of *Fusarium oxysporum* transformed with the Gus gene to study fungal activity during root colonization. Similarly, Lo et al. (1998) used a strain of *Trichoderma harzianum* transformed with the same gene to study its mycoparasitism of *Rhizoctonia solani* in rhizo- and phyllospheres.

In situ PCR entails performing PCR within fixed intact cells or tissues followed by *in situ* hybridization; thus, it is a cultivation-free and DNA extraction-free technique that allows direct correlation of morphology with DNA sequence information. One of the few applications to fungi has been by Bindslev et al. (2002), who amplified the single-copy gene *bka1* in fixed *Blumeria graminis* spores and mycelia in order to confirm that this gene (isolated from infected leaves) actually originated from *B. graminis*.

Immunological techniques can be highly specific and very powerful, but developing antibodies is costly and testing their specificity is laborious. The use of highly specific *monoclonal antibodies* to detect, quantify, and visualize plant pathogenic fungi (e.g., *Pythium*, *Phytopthora*, *Sclerotinia*, *Rhizoctonia*) in soils has been reviewed by Dewey et al. (1997). The extraradical hyphae of arbuscular mycorrhizal fungi have been assayed by direct immunofluorescence using fluorescein-conjugated polyclonal antibodies (less specific and more cross-reactive than monoclonal antibodies) raised against spores of individual genera (Egerton-Warburton and Allen, 2000).

11.5 CONCLUSIONS AND PERSPECTIVES

The use of molecular tools to detect fungal strains of plant or animal pathogens is now a routine task in many private and public laboratories. Using molecular tools, all fungal life stages are accessible without the need for cultivation. Thus, the diverse array of today's DNA-based methods has uncovered diverse new taxa throughout the true fungi (e.g., Redecker et al., 2000; Koufopanou et al., 2001; Berch et al., 2002; Schadt et al., 2003), and it has also paved the way for the characterization of previously poorly understood fungi and recognition of their distribution and ecological importance (e.g., cryptic mycorrhizal fungi such as *Tomentella*, *Thelephora*, and *Sebacina*). Further growth and improvement of the public databases will greatly accelerate these trends. Undoubtedly, fungal molecular ecology will drive exciting conceptual developments as well, as many critical areas of fungal ecology still remain largely unexplored in nature: fitness measures, active biomass, genet and ramet dimensions and connectedness, dispersal and gene flow, the dynamics of fungal competition, fungivory, symbiosis and mycoparasitism, and the relationship between phylogenetic and functional diversity. To these ends, novel methodologies, experimental designs, automation, and bioinformatics will be essential. A glimpse of this future is already visible in molecular analysis combined with functional methods (i.e., immunocapture of nucleotide analogs — Borneman, 1999; stable isotope probing —

Morris et al., 2002) and genomic functional analysis without the need for cultivation or amplification (Wellington et al., 2003).

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12

Analytical and Experimental Methods for Estimating Population Genetic Structure of Fungi

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12.1 INTRODUCTION

Fungi constitute one of the most diverse groups of eukaryotes on the planet. Their biological characteristics differ greatly from those of animals and plants. For example, the majority of ascomycetes are haploid and can transmit their genetic information to the next generation through sexual reproduction, asexual reproduction, or both. Many fungi have short generation times and wide geographical distributions, are easy to handle in large numbers, and can be experimentally manipulated without ethical or other constraints. These characteristics make fungi especially tractable as experimental organisms to test fundamental hypotheses in population and evolutionary biology. In addition, fungi cause many diseases that affect agriculture and human health. Thus, improved knowledge of the population genetic structure and evolution of fungi can increase our understanding of how virulence emerges and spreads among local populations and may have practical applications for controlling infectious diseases (Bull, 1994).

Empirical studies in population genetics usually begin by describing population genetic structure, i.e., the amount and distribution of genetic variation within and among populations, based on a limited number of individuals sampled from natural populations. To accurately and efficiently detect genetic variation, the genetic markers used in population genetic studies should be reliable and variable and should fit to the general assump-

tions of population genetic models (Brown, 1996). Empirical studies of fungal population genetics have lagged behind those for other eukaryotes because of the lack of morphological genetic markers. The advent of molecular marker technologies in the last 20 years has made empirical studies of fungal population genetics possible. Molecular marker technologies provide a large number of highly variable markers, making it possible to rapidly differentiate almost all levels of genetic variation in fungal populations. Correct and efficient detection of genetic variation also requires that sample sizes are large enough and sample strategies are appropriate (McDonald, 1997), to ensure that fungal samples included in the analysis are a fair reflection of true populations.

However, describing population genetic structure is only the initial step for population genetic studies of fungi (McDonald, 1997). The ultimate goals are to understand the evolutionary history of a population, to reveal the processes and mechanisms through which evolutionary changes have occurred, and to predict the evolutionary potential of fungal species by scrutinizing the evolutionary signals recorded in the current population genetic structure. These goals cannot be achieved solely through direct observation of fungal populations and must rely on rigorous statistical inference. The ready availability of powerful desktop computers and sophisticated analytical software has made it increasingly easy to compute population genetic parameters and develop complex evolutionary models. But the choice of appropriate analytical tools is an important issue for all population geneticists. All analytical approaches developed for estimating population and evolutionary parameters have advantages and disadvantages, depending on specific genetic assumptions. The choice of analytical methods should be based on the genetic properties of the marker system and the questions being addressed. Choosing an inappropriate analytical method could lead to false inferences regarding population genetic parameters and the evolutionary history of fungal species or populations.

In this chapter, we will focus our discussion on the properties of mathematical and statistical approaches currently used by mycologists and plant pathologists to estimate population genetic structure and to infer migration and recombination of fungal species. We also point out some problems associated with these mathematical and statistical methods, as well as some solutions. Analytical methods for inferring mutation rates, effective population sizes, and natural selection are not covered, as they are not the focus of current population genetics studies in fungi. Issues relating to the choice of genetic markers and sampling strategies have already been thoroughly discussed in other reviews (e.g., Brown, 1996; McDonald, 1997). To limit the length of this discussion, we will keep the use of mathematical formulae to the minimum. Interested readers are advised to look at cited publications and textbooks (e.g., Weir, 1996) for additional details.

12.2 EVOLUTIONARY FORCES DETERMINING POPULATION DYNAMICS OF FUNGI

12.2.1 Mutation

Mutation is the ultimate source of genetic variation, creating new DNA sequence variation upon which other evolutionary forces act (Freeman and Herron, 2001). Mutation results from base pair substitutions, small or large deletions, transposition events, and structural alteration of chromosomes. Mathematical models show that in a finite population, the probability that an allele will be lost after one generation of reproduction is $(1 - f_i)^{2N_e}$ for diploid fungi and $(1 - f_i)^{N_e}$ for haploid fungi, where f_i is the frequency of allele i and N_e is the effective population size. Thus, the ancestral lineage of any allele can be lost through random genetic drift in each generation of reproduction. After many generations, all

ancestral alleles except one will eventually disappear from the population. Therefore, in the absence of mutation, all populations will eventually become fixed for the same allele/trait and no further evolution will occur for this gene.

Although mutation creates new alleles in fungal populations, the initial frequencies of the new mutants are usually very low. In the infinite allele model, the initial frequency for a new mutant allele is $1/2N_e$ for diploid fungi and $1/N_e$ for haploid fungi, as this model assumes each allele emerges through a unique mutation. The frequency of a new mutant can be increased through recurrent mutation, but this is a slow process. For example, at least 10,000 generations are needed to increase the frequency of a recurrent mutant to a frequency of 1%, assuming a mutation rate of 10^{-6} and no reversion. Thus, the principal forces determining whether a mutant persists in a fungal population are natural selection and effective population size.

12.2.2 Migration

Migration (gene flow), here defined as the exchange of genetic information among geographic populations through the movement of gametes or individuals from one place to another (Slatkin, 1987), plays many roles in the population genetic structure and evolution of fungal species. First, like mutation, migration serves as a source of genetic variation. Because mutation is usually a rare event, a specific mutant allele may arise in one fungal population but not in others. Migration increases the genetic variation of local fungal populations by introducing novel alleles and allele combinations from neighboring populations. Evolutionary theory suggests that geographic isolation is one of the main steps leading to speciation. Regular migration acts as a constraining force on evolution by homogenizing the gene frequencies among geographically separated fungal populations, thus preventing the accumulation of population differences that could lead to speciation. On the other hand, occasional exchange of migrants can accelerate the evolutionary process by spreading superior genes or gene combinations to neighboring populations (Lande, 1984). In agricultural systems, migration is considered a critical factor for promoting pathogen evolution by spreading new virulence and fungicide resistance alleles among fungal populations across a large geographical area (McDonald and Linde, 2002).

The agents of migration in fungi can be mycelium, sexual spores, or asexual spores, as well as fertilizing agents such as microconidia and spermatia (Burnett, 2003) carried by wind, water, or insects. The principal determinants affecting migration rate of a fungal species are the type of migration unit and the mechanism used for dispersal. Fungal species dispersed by rain splash or free-water movement usually have lower potential for long-distance migration than fungal species carried by wind or insect vectors. For some fungi, such as rusts, migration units (spores) can be carried by air currents for thousand of kilometers (Watson and de Sousa, 1983), leading to intercontinental migration. Human activities, such as movement of contaminated machinery and international travel or trade, might also have a substantial impact on migration for many fungi.

12.2.3 Mating Systems

Fungi exhibit a wide array of recombination strategies. Recombination in fungi can occur through both meiosis and mitosis. Meiotic recombination usually involves the fusion of genomes donated by two opposite mating types during sexual reproduction while mitotic recombination does not involve sex. Heterokaryosis and parasexuality may provide additional mechanisms of recombination for fungal species (Burnett, 2003). Mating systems affect recombination rates in populations and play a key role in the dissipation of linkage disequilibrium. Populations exhibiting little or no recombination are expected to show a significant degree of nonrandom association among unlinked alleles. In contrast, large

recombining populations are expected to exhibit random associations among neutral loci as a result of the reassortment of unlinked genes during meiosis (Hartl and Clark, 1997). When coupled with selection, recombination can have a large impact on genetic variation (Braverman et al., 1995; Nordborg et al., 1996) and population subdivision (Felsenstein, 1974; Zhan and McDonald, 2003). First, recombination increases genetic variation in local populations by creating new genotypes through reshuffling existing genes. Even a small number of outcrossing events can generate a significant amount of genotype variation. In plants, for example, Kannenbe and Allard (1967) showed that a single outcross resulted in a significant number of new genotypes. Second, recombination can increase genetic variation by creating new alleles through intragenic rearrangement. Third, recombination increases genetic variation of local populations (Braverman et al., 1995; Nordborg et al., 1996) and decreases the level of population subdivision (Felsenstein, 1974; Zhan and McDonald, 2003) by limiting the effects of hitchhiking or background selection on linked alleles at other loci.

12.2.4 Natural Selection

The targets of natural selection are phenotypes, not genotypes. For selection to occur in natural populations, three conditions must be satisfied. First, individuals in natural populations must differ in their ability to survive and reproduce. Second, more offspring must be produced than can survive and reproduce. Third, phenotypes with higher fitness are present in excess at reproductive age and, therefore, overcontribute to the gene pool of the following generations.

While the conditions needed for selection to occur appear simple, the effects of natural selection on the genetic dynamics of fungal populations are complex. Natural selection can increase or decrease the level of genetic variation and population differentiation, depending on the types of natural selection operating on natural populations (Hartl and Clark, 1997). Directional selection favoring the same phenotypes in populations inhabiting different environments may lead to a rapid decrease in genetic variation of fungal populations while homogenizing allele frequencies among geographically isolated populations. On the other hand, balancing selection tends to increase the amount of genetic variation either through selection for rare alleles (frequency-dependent selection) or by overdominance (heterozygote advantage) in diploids or dikaryotic fungi. Divergent selection for different phenotypes among genetically isolated fungal populations may also lead to population subdivision.

Selection may play a more significant role in the population genetic dynamics of plant pathogenic fungi (Leung et al., 1993), primarily due to cultural practices, such as monoculture, frequent changes in host populations, and intensive applications of fungicides in agricultural ecosystems. Directional selection for corresponding virulent or fungicide-resistant mutants in a fungal population can rapidly change the genetic structure of fungal populations, causing the loss of effectiveness of new resistance genes or new fungicides in just a few years. Natural selection is expected to be more efficient at changing allele frequencies in haploid fungi than in diploid or dikaryotic fungi.

12.2.5 Genetic Drift

Genetic drift shares many evolutionary features with natural selection. Both directional selection and genetic drift are the principal evolutionary forces leading to fixation of rare mutant alleles. And both processes can purge genetic variation in local fungal populations and generate differentiation among subpopulations. Genetic drift is a stochastic process that affects entire genomes, while natural selection is a determinative process and affects only loci it directly acts on or is strongly associated with.

No natural populations are exempt from genetic drift. The degree of genetic drift in a fungal population is determined by its effective size. Genetic drift is more severe in small than in large populations. Thus, fungal populations with large effective sizes tend to have higher genetic variation, as more alleles can emerge through mutation and fewer alleles will be lost due to random drift (Kimura, 1983). In agricultural ecosystems, genetic drift is expected to play a more important role in the spatial and temporal genetic structure of fungal pathogen populations due to large fluctuations in fungal population sizes associated with epidemic cycles.

In small or repeatedly bottlenecked populations, genetic drift may cause accumulation or random fixation of deleterious mutations, resulting in a decline in overall population fitness. Such fitness declines can further reduce effective population size and accelerate the accumulation of additional mutations, leading to extinction of a species or population.

12.3 MATHEMATICAL AND STATISTICAL METHODS TO ESTIMATE POPULATION AND EVOLUTIONARY PARAMETERS

12.3.1 Genetic Variation

In population genetic studies of fungi with mixed sexual and asexual reproduction, both gene variation and genotype variation must be considered. *Genotype* here refers to genetically distinct individuals, often defined in fungi based on unique DNA fingerprints. The amount of gene variation in fungal populations is measured by the numbers and frequencies of alleles at individual loci and is affected by population age, migration, mating system, population size, and natural selection. For example, old populations tend to have higher amounts of gene variation due to the accumulation of mutations over time. On the other hand, the amount of genotype variation in a fungal population is affected mainly by mating and reproductive systems. Populations exhibiting largely asexual reproduction tend to have low genotype variation arrayed as a limited number of clonal lineages, whereas sexual populations are expected to exhibit a high degree of genotype variation. Genotype variation is an important population parameter only for organisms with clonal reproduction or facultative sexual reproduction, such as fungi. In obligate sexual organisms such as mammals, each individual (except identical twins) in a population has a unique genotype, and genotypic diversity should always be at its theoretical maximum.

12.3.1.1 Estimating Gene Variation

Several approaches have been used to measure and compare the amount of gene variation in fungal populations. One approach is to measure the proportion of polymorphic loci across the genome (e.g., Carter et al., 2001; Lewinsohn et al., 2001; Chen et al., 2002; Plante et al., 2002; Flier et al., 2003). Polymorphic loci are defined as those where the most common allele is present at a frequency of less than 0.95 or 0.99 (Hartl and Clark, 1997), depending on the preference of the researchers. Previous studies indicate that the distribution of genetic variation among loci for most organisms follows an L shape, where the majority of loci in the genome show little or no variation (Nei, 1987). This implies that to ensure 95% probability of detecting polymorphism for a less variable locus, at least ~30 diploid individuals should be assayed for the 0.95 criterion of polymorphism and ~150 for the 0.99 criterion. These numbers increase to ~60 and ~300, respectively, for haploid fungi. Many sample sizes used in studies of fungal population genetics are much smaller than these expectations and, thus, are likely to underestimate the level of polymorphism.

A second approach is to measure allelic richness, i.e., the mean number of alleles at each locus (e.g., Elias and Schneider, 1992; Boisselier-Dubayle et al., 1996; Huss, 1996; Purwantara et al., 2000; Johannesson et al., 2001a; Zhan et al., 2003). When calculating allelic richness of populations, all loci should be taken into account, including monomorphic loci. Measures of allelic richness should only be applied to data generated using multiallelic markers such as restriction fragment length polymorphisms (RFLPs) and microsatellites. For data generated with biallelic polymerase chain reaction (PCR)-based markers, for example, amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD), measures of allele richness are limited because these markers have a maximum of only two possible alleles. Both theoretical and experimental studies have shown that allelic richness is very sensitive to sample sizes (Nei et al., 1975; Leberg, 1992; Spencer et al., 2000), especially for highly variable loci. In the long term, allelic richness is thought to be a more accurate reflection of evolutionary potential of populations than other measures of gene variation (e.g., Petit et al., 1998).

A third approach is to measure the amount of heterozygosity (e.g., Hantula et al., 1998; Vainio et al., 1998; Hogberg and Stenlid, 1999; Smith and Sivasithamparam, 2000; Johannesson et al., 2001b). The amount of heterozygosity is often presented for a single locus or as an average across several loci and can only be used for diploid or dikaryotic fungi.

The fourth and most widely used approach to measure gene variation in fungal populations, however, is Nei's (1973) gene diversity (e.g., Goodwin et al., 1994; Leuchtmann and Schardl, 1998; Rosewich et al., 1999; Pimentel et al., 2000; Gagne et al., 2001; Douhan et al., 2002; Mahuku et al., 2002; Zhan et al., 2003). Nei's gene diversity is a joint function of allele richness and allele frequency. It measures the potential amount of heterozygosity in random mating diploid populations. Nei's gene diversity can be used to measure gene variation for any organism as long as allele frequencies can be determined. When estimating gene diversity of a fungal population, data from several loci should be averaged, including monomorphic loci. Like allelic richness, measures of gene diversity are affected by sample sizes, but are less sensitive than allelic richness (Nei et al., 1975; Leberg, 1992; Spencer et al., 2000).

After gene variation has been calculated for a fungal population, it is common to compare it with the gene variation of other populations and even other fungal species, quantitatively or qualitatively. Results from these types of comparisons are routinely used as arguments to support hypotheses of genetic drift or natural selection, as well as to indicate the evolutionary potential of a particular fungal population or species. When comparing the amount of gene variation in fungal populations, several parameters have to be considered, especially when comparing data from different laboratories, including:

1. What types of genetic markers were used. The types of genetic markers chosen have a strong impact on the estimate of gene variation. The maximum genetic variation, in terms of Nei's gene diversity, is $1 - 1/k$, where k is the number of alleles. For data generated with biallelic PCR markers, such as RAPD and AFLP, the maximum number of alleles is two and the maximum value of Nei's gene diversity is 0.50. When data are based on multiallelic markers, such as RFLPs and microsatellites, the number of alleles at one locus can be very large and gene diversity can be close to the theoretical maximum of 1.0. Therefore, comparisons of gene variation can be largely meaningless if different genetic marker systems are used.
2. Whether monomorphic loci are included. In many population genetic studies of fungi, researchers have considered only data from polymorphic loci. For RFLP and microsatellite markers, these loci are chosen randomly from a

genomic library during a prescreening procedure and may represent the most variable parts of the genome. Data from less variable (monomorphic) loci are either discarded or are not included in surveys. This exclusion of monomorphic loci not only inflates measures of genetic variation of fungal species, but also makes comparisons from different laboratories difficult or impossible.

3. Whether sample sizes are the same. Unequal sample sizes are commonly encountered in population surveys, even in data sets generated from the same laboratory. As all four measures of gene variation are affected by sample size, quantitative comparisons of gene variation among fungal populations should be based on standardized sample sizes. Although some researchers have noted the relationship between gene variation and sample sizes, the implications of this relationship are apparently not widely understood, and the majority of researchers have not attempted to correct for differences in sample size. Several strategies can be used to correct for differences in sample size among populations (Leberg, 2002). In our laboratory, we have used three methods to make this correction:
   a. First, we have standardized different fungal samples with θ ($4N_eu$ for diploid organisms and $2N_eu$ for haploid organisms), the expected number of new alleles emerging through mutation each generation (Zhan et al., 2001). The θ of a fungal population was estimated from the observed number of alleles in a sample using the sampling theory developed by Ewens (1972). This strategy does not compare allelic richness directly. Rather, it compares the potential of generating new mutants under an infinite neutral allele model. By assuming constant mutation rates across populations, this strategy can also be used to compare effective population sizes among populations. A drawback of this strategy is that it requires large sample sizes to make a reasonable estimate.
   b. A second strategy is to standardize populations with resampling by taking many random subsamples of the same size from the original data set of each population and calculating gene variation as the mean value of the resamples (Zhan et al., 2003). In a global survey of the wheat pathogen *Mycosphaerella graminicola*, we found that populations from the Middle East displayed the highest amount of gene variation and we hypothesized that this region is the center of origin of this fungus. However, initially we did not know whether differences in gene variation among regional populations reflected differences in sample sizes or whether the differences were statistically significant. We used the resampling strategy to distinguish between these possibilities. For each resampling replication, the total number of alleles and their frequencies were recorded from a random sample of the same size as the smallest population and the gene diversity was calculated. This procedure was repeated 100 times. The mean and variance for gene diversity and allele number in each population were calculated and used for a t-test. When using random resampling strategies to standardize sample size, it is important to standardize them according to the population with the smallest sample sizes. Reducing the larger samples to the mean of all populations, for instance, would reduce but not eliminate bias. The advantage of this procedure is that it provides a statistical test for the difference while standardizing sample sizes.
   c. A third method is to standardize estimates of allelic richness (Zhan and McDonald, 2003) with a sample coverage method by estimating the number of undetected alleles according to sample sizes and the frequency distribution of alleles (Huang and Weir, 2001). This method estimates the total number

of alleles in a population regardless of sample size and is a good indicator of true gene variation.

12.3.1.2 Estimating Genotype Variation

Many methods have been used to estimate genotypic diversity in fungal populations. These methods can be grouped roughly into three categories. As explained below, all of these have some inherent problems, and a better estimator of genotype diversity would represent a significant advance for fungal population studies.

The first category is based on Nei's measure of gene diversity (Nei, 1973) and its analogs. This approach has two problems. The first is that Nei's gene diversity measures the expected degree of heterozygosity assuming populations are random mating. As mentioned earlier, genotypic diversity in obligate sexual organisms should always be at its maximum. Any departure from this expectation can be due to nonrandom mating or asexual reproduction. Therefore, Nei's gene diversity is not a good measure of genotypic diversity. When we compared different analytical methods, we found that Nei's method usually gave the highest estimates of genotypic diversity (J.Z., unpublished results), suggesting that this method biases the estimates upward. The second problem is that heterozygosity measures only a part of genotype diversity (Gregorius, 1978). Homozygosity does not necessarily decrease the amount of genotypic diversity in populations. For example, in populations undergoing obligate sexual reproduction, all individuals usually have distinct multilocus genotypes and contribute equally to the level of genotype variation, regardless of whether they are homozygous or heterozygous for particular loci.

The second category is represented by Stoddart and Taylor (1980) using Kimura and Crow's (1964) effective number of alleles as an index of genotypic diversity. This index of genotypic diversity has a minimum value of one and maximum value of n, where n is the sample size. This measure is strongly associated with sample size, making comparisons among populations of unequal sample size impossible. This problem can be circumvented by normalizing estimated genotypic diversity against sample size, resulting in the percentage of the theoretical maximum (Chen et al., 1994), but normalizing is thought to work well only when populations have high diversity (Grünwald et al., 2003). Furthermore, the index of Stoddart and Taylor (1980) emphasizes the most common genotypes contributing to the genotypic diversity of populations while underrepresenting the contribution of genotypes that are rare.

The third category is based on the Shannon–Wiener index (Shannon and Weaver, 1949; Muller et al., 2002; Sigler and Turco, 2002), also called the Shannon (Steffenson and Webster, 1992; Kolmer 1993; Purwantara et al., 2000) or Shannon–Weaver (e.g., Burdon and Jarosz, 1992; Meijer et al., 1994; Garbeva et al., 2003) index. Because the first version of the Shannon–Wiener index was published in 1948 (Shannon, 1948), a year before publication of the book *The Mathematical Theory of Communication*, coauthored by Shannon and Weaver (1949), and is built upon the work of Wiener (1948), some researchers (e.g., Spellerberg and Fedor, 2003) are against using the names Shannon or Shannon–Weaver index.

The Shannon–Wiener index, ranging in value from zero to infinity in theory, was originally developed for telecommunication to estimate the average uncertainty in predicting letters of a message, but has been widely used in many fields, including geography (e.g., Haines-Young and Chopping, 1996; Nagendra, 2002), ecology (e.g., Floder et al., 2002; Stampe and Daehler, 2003), and population genetics (e.g., Drenth et al., 1993; Kwon and Morden, 2002). This index emphasizes the measure of species (genotype) richness (Hurlbert, 1971) and the importance of species or genotypes with a low or rare frequency

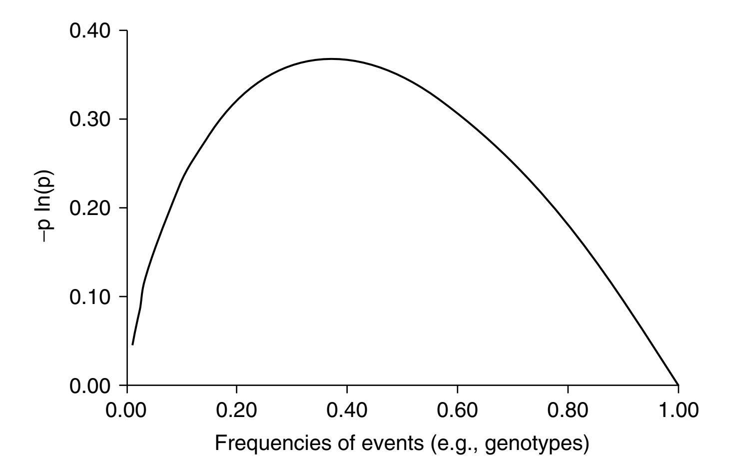


Figure 12.1 The contribution of an event's frequency (genotypes, species, etc.) to the estimate of the Shannon–Wiener index.

(Figure 12.1). This index is also affected by sample size and suffers problems similar to the Stoddart and Taylor index when normalized according to sample size (Grünwald et al., 2003). More discussion on the estimation of genotypic diversity and its associated problems can be found in a recent publication (Grünwald et al., 2003).

12.3.2 Population Subdivision

When genetic data are available for several fungal populations, it is natural to ask what degree of population subdivision exists among the populations surveyed. Population subdivision, here defined as the difference in allele frequencies among subpopulations, can be generated by genetic drift and natural selection. In the absence of sufficient gene flow among populations, stochastic changes in allele frequencies among fungal populations can lead to random fixation of neutral alleles, leading to nonadaptive differentiation (Wright, 1938). On the other hand, divergent selection for various ecological or physiological characters among genetically isolated fungal populations may lead to adaptive population subdivision. Population subdivision caused by natural selection usually is limited to the loci directly affected by natural selection and closely linked genes, unless fungal populations are dominated by asexual reproduction.

The simplest way to measure the magnitude of subdivision among fungal populations is by testing for homogeneity in gene frequencies using two-dimensional contingency χ^2 tables (e.g., Rosewich et al., 1998; James et al., 1999; McDonald et al., 1999; Tenzer and Gessler, 1999; Salamati et al., 2000), such as introduced by Workman and Niswander (1970). This type of chi-squared test has (r – 1) × (c – 1) degrees of freedom, where r refers to the number of alleles at a locus and c refers to the number of fungal populations. The *p* values generated by the contingency chi-squared test provide implicit statistical information on whether the fungal populations compared differ in their allele frequencies and at what levels. Furthermore, the sensitivity of the test increases with sample size. Because small populations are more prone to sampling error, this property provides a way to autocorrect the potential type I error associated with small samples.

Three problems may be encountered when using the contingency χ^2 test. First, when some alleles have small expected values under the hypothesis of equal frequencies across populations, it is necessary to combine the less frequent alleles into one category. Other-

wise, the resulting χ^2 value may be too large, causing a false rejection of the null hypothesis. Many statisticians recommend that χ^2 should not be performed if the expected count in the cells is less than five, but this criterion is hard to realize unless sample sizes are large and loci are moderately variable. In our laboratory, we usually combine into a single category alleles with frequencies lower than 0.05 for studies with large sample sizes (>50 individuals per population) and 0.10 for studies with small sample sizes (<30 individuals per population). Second, a contingency χ^2 test is conducted for each locus, and statistical inference regarding population subdivision may not be consistent across randomly chosen loci. In many experimental studies in fungal population genetics, it is common to find that the null hypothesis of equal allele frequency across populations is rejected for one or two loci, but not for other loci. This inconsistency could be expected if the loci chosen have undergone different evolutionary processes. For example, some of the loci could be under selection or linked to selected loci, while others are selectively neutral. In this case, further tests for neutrality of suspect loci may be required. On the other hand, information from several loci can be joined to give a combined χ^2 value. This combined χ^2 value has $(c - 1) \sum (a_i - 1)$ degrees of freedom, where c is the number of fungal populations surveyed and a_i is the number of alleles at the *i*th locus. Third, when multiple fungal populations are compared, rejection of the null hypothesis of equal frequencies indicates only that there is some degree of genetic differentiation among the populations surveyed. The rejection could be caused by one or a few extreme populations, and the contingency χ^2 test would not identify the responsible populations. To identify the extreme populations, more tedious procedures such as pair-wise comparisons between fungal populations may be needed.

Another approach widely used to measure subdivision among fungal populations is the fixation index, F_{ST} and its analogs (e.g., Carlier et al., 1996; Milgroom et al., 1996; Purwantara et al., 2000; Douhan et al., 2002; Garcia et al., 2002; Johannesson et al., 2001b; Kauserud and Schumacher, 2003; LoBuglio and Taylor, 2002; Urbanelli et al., 2003). The term *fixation* was first coined by Wright (1921) to quantify the reduction of heterozygosity compared with a random mating population. The deficiency of heterozygosity in a population could result from nonrandom mating within local populations. It also could result from differences in allele frequencies among populations due to cryptic population subdivision. If two subdivided populations are combined in an analysis, there often is an excess in homozygosity (reduction of heterozygosity) called the Wahlund effect. The degree of this excess reflects the amount of genetic differentiation between the two subpopulations. The excess homozygosity in the combined population will reduce to that expected after only one generation of random mating. This reduction in homozygosity after one generation of random mating is called the Wahlund principle. Wright (1951) used the formula $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$ to partition the fixation index of a subdivided population, where F_{IT} is the total reduction of heterozygosity (also called the inbreeding coefficient) in subdivided populations, and F_{IS} and F_{ST} are the lack of heterozygosity due to nonrandom mating within local populations and to population subdivision, respectively. F_{ST} has a value between 0 and 1. When F_{ST} equals one, it indicates that populations are completely isolated, and when F_{ST} equals zero, it indicates the populations are identical.

One advantage of using F statistics to measure the level of population differentiation is that F_{ST} also provides a convenient way to estimate gene flow, provided that populations are in drift–migration equilibrium and mutation is negligible. However, the F statistics also have a few deficiencies. First, F_{ST} does not have a statistical property. To test whether an estimate is statistically significant, further analysis using a Fisher exact test or permutation is required. In practice, it is common to conclude that populations show little differentiation if F_{ST} is less than 0.05, moderate differentiation if F_{ST} is between 0.05 and 0.15, large differentiation if F_{ST} is between 0.15 and 0.25, and very large differentiation

if F_{ST} is larger than 0.25, but these values are arbitrary. Second, though F_{ST} can be calculated for any population if there are two alleles at one locus, in the presence of multiple alleles, the formula $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$ is no longer valid unless there is no selection (Nei, 1965). Third, F_{ST} can only be used for diploid organisms. For haploid fungi, no measure of heterozygosity is possible. Fourth, F_{ST} does not take sample sizes and number of subpopulations into account.

To overcome these problems, several analogs of F_{ST} have been developed and widely used to measure population subdivision. One of these analogs is G_{ST} (Nei, 1972, 1973). G_{ST} is an averaged F_{ST} over alleles and pairs of populations. Nei (1973) stated that G_{ST} can be applied to any populations without assumptions regarding the pattern of evolutionary forces, such as mutation, selection, and migration, and to sexual or asexual organisms of any ploidy as long as allele frequencies can be estimated. Furthermore, G_{ST} can be used to quantify population subdivision across many hierarchical levels as long as an appropriate sampling strategy is used. Like F_{ST}, G_{ST} (Nei, 1972, 1973) does not have a statistical property and further analysis is required to obtain a statistical inference. It also does not explicitly take into account differences in sample sizes and number of subpopulations (Nei and Chesser, 1983).

Another F_{ST} analog is θ_{ST}, proposed by Cockerham (1969, 1973) and Weir and Cockerham (1984). θ_{ST} is estimated from each allele separately. When there are more than two alleles present at a locus, information from the different alleles can be combined by taking their geometric means (Weir and Cockerham, 1984). θ_{ST} emphasizes the effects of sample sizes and number of subpopulations on the estimation of population subdivision. This statistic assumes that populations are random mating and have equal effective size. Unlike G_{ST}, values of θ_{ST} are not always positive. A negative θ_{ST} value indicates that the populations are so similar in gene frequencies that no population subdivision can be detected, but the negative value cannot be used to estimate the amount of gene flow.

More recently, analysis of molecular variance (AMOVA) based on the conventional theory of analysis of variance (Excoffier et al., 1992) has become widely used to study spatial population structure of fungi (e.g., Hellgren and Hogberg, 1995; Hamelin, 1996; Norden, 1997; Pimentel et al., 2000; Vainio and Hantula, 2000; Viji et al., 2001; Barrins et al., 2002; Coates et al., 2002; Jamaux-Despreaux and Peros, 2003; Kauserud and Schumacher, 2003; Samils et al., 2003; Urbanelli et al., 2003). This analysis produces estimates of variance components by partitioning the total sum of the squared distances between all pairs of haplotypes hierarchically and generates ϕ statistics. The method can accommodate various types of genetic assumptions on the evolution of populations (Excoffier et al., 1992) and can provide a statistical test on the significance of the differentiation index. But the method is computation-intensive. AMOVA relates all differences among DNA haplotypes to step-wise mutation events and estimates evolutionary divergence (distance) based on the number of mutational steps between haplotypes. AMOVA was developed for analysis of molecular data from nonrecombining regions, such as mitochondrial genomes, and is probably valid for asexual fungal populations. Some researchers have used AMOVA to analyze molecular data from recombining regions, thus invalidating the fundamental assumptions of the method.

Using F statistics (F_{ST} and its analogs) to quantify population subdivision assumes a K allele or infinite allele mutation model. These models state that mutation events should be rare and independent of the prior states of ancestral alleles so that any genetic similarity among natural populations can be attributed to historical association or gene flow. These assumptions may be reasonable for RFLP, AFLP, and RAPD loci, but they may not apply to many microsatellite loci. Experimental studies from humans and other eukaryotic species have shown that mutation rates are very high for many microsatellite loci (e.g.,

Udupa and Baum, 2001) and the size of new mutant alleles depends on the size of their ancestral alleles (for a review, see Li et al., 2002). Therefore, F_{ST} and its analogs may not be appropriate for estimating population subdivision of microsatellite data and may overestimate the actual level of genetic similarity among populations. In this case, Slatkin (1995) proposed to use R_{ST}, a quantity analogous to F_{ST}, but which takes into account differences in allele lengths. Results from computer simulations showed that R statistics performed better for microsatellite data than F statistics (Slatkin, 1995).

Many researchers also use genetic distance to measure the similarity between fungal populations (e.g., Weir et al., 1998; James et al., 1999; Six and Paine, 1999; Lakrod et al., 2000; Valverde et al., 2000; Vandemark et al., 2000; Bock et al., 2002; Hurtado and Ramstedt, 2002; Plante et al., 2002; Says-Lesage et al., 2002). There are several types of genetic distance, but Nei's genetic distance (1972, 1978) is most commonly used. Nei's genetic distance is defined as $D = -\ln I = -\ln [J_{XY}/(J_X\ J_Y)]^{0.5}$, where I and J_{XY} are the normalized genetic identity and the probability of identity by descent between populations X and Y, and J_X and J_Y are the probability of identity by descent within each population, respectively. This quantity is similar to G_{ST} in many ways. It measures the accumulated nucleotide substitutions per locus between populations. Because genetic distance is thought to be linearly related to the time since populations diverged from the same ancestor, the advantage of this quantity is that it can be used to calculate divergence time between two fungal populations, provided that all assumptions for G_{ST} are satisfied and the rate of nucleotide substitution is constant over time. Furthermore, although Nei's genetic distance has been used primarily to measure genetic similarity of populations within species, it can also be used to measure similarity between distantly related species (Nei, 1972).

To estimate genetic distance, data from several loci can be combined, based on either the geometric mean of I or the arithmetic means of Jxy, Jx, and Jy. How to weight different loci is dependent on the level of genetic similarity between the compared fungal populations and the evolutionary rates among the loci considered. Nei (1974) proposed using the geometric mean of I to weight different loci if the evolutionary rates among loci were different and genetic identity (I) between populations was high. Otherwise, the arithmetic mean of the J values should be used. Apparently, this argument is largely ignored in empirical studies of fungal population genetics, possibly due to the fact that evolutionary rates of loci used are not available in most cases. Because many molecular analyses of eukaryotic genomes have revealed that the evolutionary rates vary across genomes (Kimura, 1983; Kusumi et al., 2002), we believe that researchers should use the geometric mean to weight genetic distance among loci in cases where evolutionary rates of loci are unknown and the populations compared are highly similar. When data from different markers are combined, it is important to include all loci, including monomorphic loci (Nei, 1972). However, the majority of researchers, including ourselves (Zhan et al., 2003), have included only polymorphic loci. Excluding monomorphic loci from analysis of genetic distance undoubtedly overestimates the genetic differences between fungal populations.

12.3.3 Inference of Evolutionary Forces

Migration, the amount of gene flow among fungal populations, is usually estimated indirectly based on the distribution of allele frequencies among subpopulations. Currently, two indirect methods are widely used to derive the amount of migration among fungal populations. One of them is Wright's F_{ST} statistic for estimating the standardized variance of allele frequencies among local populations (Wright, 1951). Wright (1951) showed that in an infinite island model, the level of population subdivision is a function of effective population size and the average rates of migration among subpopulations. If the migration rate is small and the effective population size large, the relation between population

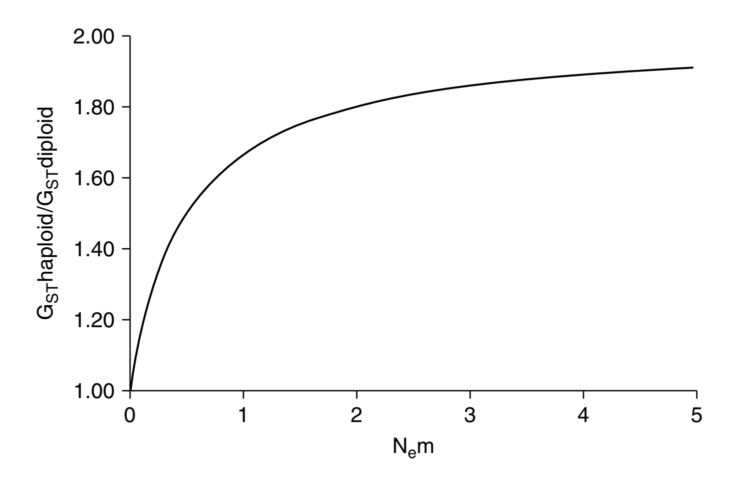


Figure 12.2 The ratio of G_{ST} values between haploid and diploid fungi as a function of gene flow.

subdivision and migration can be expressed as $F_{ST} = 1/(4N_em + 1)$ for diploid organisms, where N_e is effective population size and m is the migration rate. For haploid organisms, such as many ascomycetes, as well as mitochondrial or plastid genes, the formula can be modified to $F_{ST} = 1/(2N_em + 1)$. This expression indicates that if N_em is large, haploid fungi are approximately two times more sensitive to population subdivision (i.e., through genetic drift or natural selection) than diploid fungi (Figure 12.2). If N_em is small, diploid and haploid fungi are essentially equally sensitive to population subdivision. If mitochondrial or plastid genes are uniparentally inherited and $N_{males} = N_{females} = 1/2\ N_e$, as in the heterothallic fungus *M. graminicola* (Zhan et al., 2002), the above formula can be further modified to $F_{ST} = 1/(N_em + 1)$, suggesting that mitochondrial or plastid genes are more sensitive to population subdivision than nuclear genes.

Another method depends on the frequencies of private alleles, alleles present in only one population (Slatkin, 1985). The private allele method is based on the finding that the average frequencies of private alleles [p(1)] are a simple function of N_em. The average amount of migration per generation can be expressed as $\ln[p(1)] = a \ln (N_em) + b$, where a and b are constants. This method is very convenient but has some drawbacks. First, constants a and b are known only for sample sizes of 10, 20, and 50 (Slatkin, 1985). For other sample sizes, the two constants have to be standardized accordingly. Second, the method cannot be used for species that exhibit high migration because the relationship between $\ln[p(1)]$ and $\ln(N_em)$ is not linear when N_em is larger than five. And third, only private alleles are included in the analysis, while the remaining information is discarded.

Measurement of migration rates with indirect methods assumes that migration occurs at a constant rate over a large number of generations, and populations are at drift–migration equilibrium. Indirect methods lead to the average number of individuals that are successfully incorporated into the breeding system of the resident population (Slatkin, 1987). One disadvantage of indirect methods is that the estimate of migration rate is always expressed as the product of effective population size and migration. Effective size is unknown for most fungal populations and is sensitive to the level of population differentiation and the geographic scale over which the fungal population is sampled (Chesser et al., 1993; Whitlock and Barton, 1997). As a result, migration rates cannot be estimated by these indirect methods. A second disadvantage of indirect methods is that they assume equal migration rates between or among the populations and are unable to distinguish among

donor and recipient populations. A third disadvantage of indirect methods is that they indicate the long-term effects of gene flow on semi-isolated populations but cannot detect sporadic or rare migration events. The final disadvantage is that indirect methods cannot distinguish between current gene flow and historical gene flow. As an example, in some fungi, natural populations sampled worldwide have highly similar allele frequencies, suggesting that significant gene flow occurred among these populations (e.g., Zhan et al., 2003). However, it is not known whether the lack of population differentiation reflects past movements of gametes (genotypes) among the populations or whether gene flow acts as a significant evolutionary force at the present time (e.g., Zhan et al., 2003). In these cases, further analysis with maximum likelihood (Nielsen and Slatkin, 2000) may be necessary to find the time scale of migration.

Gene flow can also be measured directly. Direct measurement of gene flow may provide important insight into the role of migration on the evolution of organisms over contemporary time scales (Rannala and Mountain, 1997). While direct methods measure the number of individuals moving from one population to another, they do not necessarily reflect the number of individuals incorporated into the breeding system of local populations. As a result of local maladaptation, many immigrants may not survive and reproduce in a new environment. On the other hand, immigrants containing superior genes or gene combinations may establish and multiply, disproportionately contributing to the gene pools of the resident populations.

In fungi, experimental reports on gene flow measured with direct methods are very limited. Currently, three strategies have been used to directly measure gene flow in fungi. The first strategy, detection of identical genotypes with molecular markers, was used recently to detect gene flow in *Phytophthora infestans* and *Fusarium oxysporum* f. sp. *cubense*. Goodwin et al. (1995) assayed 130 Canadian and U.S. strains of *P. infestans* for variation in DNA fingerprints. They found that strains causing epidemics of potato late blight in the two countries during 1992 and 1993 were different from previous collections from the same countries but similar to those observed in Mexico. Koenig et al. (1997) and Bentley et al. (1998) assayed *F. oxysporum* f. sp. *cubense*, the pathogen causing Panama disease of banana, with several types of genetic markers and found that the global fungal population was dominated by a few common clonal lineages, suggesting that global gene flow occurred in this fungus. Due to the fact that only a limited number of individuals can be assayed with current molecular techniques, direct estimates of gene flow through the detection of identical genotypes are not likely to be practical for sexual fungi.

A second strategy is to identify source populations using posterior probability. A few methods (Rannala and Mountain, 1997; Zhan et al., 1998) based on this theorem have been used in both fungal and nonfungal systems to evaluate genetic differentiation among natural populations (Paetkau et al., 1995), to compare the dispersal rates between sexes (e.g., Helbig et al., 2001), to detect immigration (Rannala and Mountain, 1997), to source populations of migrant individuals (e.g., Pritchard et al., 2000; Fritzner et al., 2001; Ceresini et al., 2003), to identify the parenthood of individuals (Boyd et al., 2001), and to distinguish immigrants and recombinants (Zhan et al., 1998). The mathematical part of this approach consists of two steps. The first step involves the calculation of prior probabilities of being immigrants or residents for a particular multilocus genotype based on the allele frequencies drawn from reference populations, assuming gametic equilibria among the loci. The second step is to calculate posterior probabilities of being immigrants and residents according to the prior probabilities estimated from the first step. An individual is considered to be an immigrant if its posterior probability of being an immigrant is larger than 0.5 (Paetkau et al., 1995; Rannala and Mountain, 1997) or 0.9 (Zhan et al., 1998).

One inherent problem associated with this strategy is how to treat the alleles that are not found in a population. If an allele is not observed in a population sample, the null frequency of this allele can be replaced either by a small value or by the inverse of the number of individuals sampled from the total population (Cornuet et al., 1999). Another difficulty associated with this approach is to decide which allele frequencies should be used to estimate prior probabilities of being immigrants and residents. We used allele frequencies in earlier collections from the same population to compute prior probabilities (Zhan et al., 1998). This approach assumes that allele frequencies in the investigated populations are stable over time and requires prior knowledge of the genetic structure of the species. Alternatively, many researchers simply used allele frequencies observed in the samples.

This approach assumes that the allele frequencies deduced in the population samples are close to their true values and might work well when immigration rates are low. In this case, most individuals in the local populations are the progeny of parents from the same population. However, when immigration rates are high, many individuals in the local populations are likely to be immigrants from other populations that may have different allele frequencies. Including these immigrants in the estimation of allele frequencies of the local population, and then using these allele frequencies to compute the probabilities for multilocus genotypes produced in the local population, could introduce a bias. This problem could be overcome by an iterative procedure where the allele frequencies estimated from a sample are used to estimate prior and posterior probabilities and to assign the source population (immigrant or resident) for each genotype. The new populations are formed with this knowledge and allele frequencies are recalculated from these new populations. Only genotypes with a certain criterion (say posterior probability > 0.9) of being an immigrant are considered to belong to the immigrant population. All other genotypes are grouped into the original resident populations. This procedure can be repeated as many times as necessary until all genotypes that have potential contribution to the reproduction of a population are included in the calculation of allele frequencies in that population.

In addition to the two problems discussed previously, a significant disadvantage of these methods is that due to the lack of informative immigration rates, immigrants are assigned by using the simple ratio of the prior probabilities for each genotype produced in each assayed population. These methods assume that an individual drawn at random from any assayed population is equally likely to be an immigrant or a resident and that the likelihood of observing a particular multilocus genotype in the assayed populations is an exclusive character for each individual. Therefore, posterior methods can only detect possible immigrants but cannot estimate the rate of immigration. An advantage of maximum likelihood approaches (Zhan et al., 2000) is that they can directly estimate the rate of migration among populations by using information obtained from genetic studies of the populations and then use the estimated migration rate as a known parameter to identify individuals to their respective source populations. Maximum likelihood considers the probability of observing a particular multilocus genotype in any assayed population as the joint function of the relative prior probabilities of this multilocus genotype produced in each assayed population and the rates of migration among these populations. Coupled with DNA fingerprinting, single-locus RFLP markers, and an appropriate field design, we used this method to estimate the relative contributions of sexual reproduction, asexual reproduction, and immigration to the population genetic dynamics of the wheat pathogen *M. graminicola* (Zhan et al., 2000).

12.3.3.1 Mating Systems

A number of strategies can be used to determine the mating system in a fungal population. These methods include direct observations of anamorph and teleomorph, measuring the

amount and distribution of genotype variation, and measuring the randomness of association among alleles (Burnett, 2003). Of these, measures of associations among alleles are most commonly used to deduce mating systems of a fungal population. For diploid fungi, the best way to measure the extent of association between alleles is to test for a deviation from Hardy–Weinberg equilibrium at each individual locus. In the absence of other evolutionary forces, fungal populations are expected to reach Hardy–Weinberg equilibrium after a single generation of random mating, regardless of their population genetic structure in previous generations. Any statistical departures from Hardy–Weinberg equilibrium may provide some evidence of nonrandom mating, and the type and extent of the departure can be used to determine the types of mating systems in the fungal populations. An excess of homozygosity is a positive indicator of inbreeding or positive assortative mating, while an excess of heterozygosity may indicate that the populations are under disassortative mating. The association of alleles in diploid fungi may also be measured based on departures from gametic equilibrium. Unlike Hardy–Weinberg equilibrium, gametic equilibrium may not be achieved after one generation of random mating if the parental populations are in gametic disequilibrium, but disequilibrium will decay at the rate of 50% per generation in a random mating fungal population. Because gametic phases usually cannot be determined for dihybrids, testing for gametic equilibrium is not an efficient approach of measuring allelic associations for diploid fungi.

For haploid fungi, testing for gametic equilibrium is an efficient way to measure allelic association (e.g., Kohli and Kohn, 1998; Zhan et al., 1998). Gametic equilibrium can be tested pair-wise (Weir, 1996), either according to individual allelic pairs (allele by allele) or across all allelic pairs among pairs of loci (locus by locus). The advantage of pair-wise comparisons is that they provide information on the particular allele and locus combinations causing the potential rejection of the null hypothesis, and this knowledge can be used to identify putative parents and offspring in a population (Zhan et al., 1998).

Pair-wise comparisons are manageable when there are few alleles per locus and few loci are included. When multiple alleles and many loci are considered, the number of comparisons increases rapidly, making the analysis impractical. Furthermore, in pair-wise comparisons, the same set of data is applied to make many comparisons. Because the probability of incorrectly rejecting a null hypothesis in each test conducted at the α level is α (Milton and Arnold, 1995), the probability of at least one comparison being incorrectly rejected (type I error) is $1 - (1 - \alpha)^{k(k-1)/2}$, where α is the level of significance and k is the number of loci. For example, the probability of at least one comparison being incorrectly rejected is about 0.9 if $\alpha = 0.05$ and 10 loci are used. Thus, when the number of loci increases, the overall probability of type I error may become unacceptably high. To compensate for this problem, some correction with the Bonferoni procedure may be necessary. Instead of using the original level of significance (α), Bonferoni adjustment proposes that comparisons should be conducted at a lower, i.e., $\alpha' = 2\alpha/k(k - 1)$, significance level. The choice of α value is at the discretion of the researchers and should be chosen according to the number of tests run (Milton and Arnold, 1995). Sometimes, the numerical value of α could be set as high as 0.2. It is important to note that if is small, then $\alpha' = 2\alpha/k(k - 1)$ will be even smaller. If we try to force a small experiment-wise error (α), then α' becomes so small that it becomes very difficult to reject the null hypothesis, causing type II error.

Alternatively, the extent of gametic disequilibrium in haploid fungal populations can be measured with analysis of multilocus associations (Brown et al., 1980). The analysis of multilocus associations computes the variance of the number of heterozygous loci (S_k^2) from samples and compares the observed variance to expected values under random association. The variance of the number of heterozygous loci is a summary statistic over

all loci and allele combinations in a population, and this quantity increases as the populations deviate from completely random assortment. The null hypothesis of random mating is rejected if the observed variance of the number of heterozygous loci is beyond the higher boundary of its expected value. The analysis of multilocus association (Brown et al., 1980) is less influenced by rare allele pairs than pair-wise comparisons. One drawback of this method is that it ignores the behavior of particular allele or locus combinations. Another drawback is that this analysis is sensitive to missing data. When data sets are incomplete (missing data in some individuals), the estimated variance of the number of heterozygous loci tends to be biased downward. Brown et al. (1980) suggested that a correction factor should be added in this case. When we tested allelic associations using some incomplete data from *M. graminicola* populations according to this suggestion, we found that the hypothesis of random association was falsely rejected in several populations. We suspect that the proposed correction factor may overcompensate for the actual underestimate of S_k^2 with incomplete data and suggest using only complete data by excluding either some individuals or loci from the data set, whichever is applicable.

Although not falsifying the null hypothesis of Hardy–Weinberg or gametic equilibrium may provide supporting evidence that the investigated fungal population is random mating, the converse is not true. Many researchers use rejection of the null hypothesis as an argument for clonal reproduction or lack of sexuality, these arguments are simply wrong. The context of sexuality can include selfing, assortative mating, and infrequent outcrossing, but only sex in the form of regular and random outcrossing is expected to lead to random mating.

12.4 CONCLUSIONS

Population genetics combines biology and computational science. Improvements in computation and analytical technologies have contributed significantly to the recent progress in experimental studies of population genetics. Over the past 10 years, many sophisticated and computer-intensive analytical tools have been developed for characterizing population and evolutionary parameters and incorporated into freely accessible computer programs. Though the majority of these analytical methods were developed for diploid or sexual organisms, many of them can be adapted for haploid or asexual fungi without further modifications as long as the fundamental assumptions of the analytical methods are not violated. In this chapter, we focus our discussion only on basic analytical methods for characterizing population genetic structure of fungi. Some of these analytical methods are now routine, while others (e.g., posterior probability and maximum likelihood estimation of gene flow) are quite new and still under debate among plant pathologists and mycologists (Brown, 2000; Zhan et al., 2000), though these principles and approaches have been widely adopted by other fields (e.g., Paetkau et al., 1995; Rannala and Mountain, 1997; Pritchard et al., 2000). We hope that this chapter will stimulate deeper discussion on the tools and problems associated with analysis of population genetic data as well as provide some guidance for the next generation of fungal population geneticists. In addition, we believe that fungal population genetic studies in the future should move the focus from surveying population genetic structure to testing specific evolutionary hypotheses postulated during population surveys.

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13

Interspecific Interaction Terminology: From Mycology to General Ecology

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13.1 INTRODUCTION

Terms used to describe interspecific interactions are not clear for many reasons. Mycologists have frequently borrowed terms from general ecology to describe interspecific interactions, yet descriptions are not completely applicable to fungi in many instances. Within the field of mycology, many terms are too specific even to be applied to all fungi. Both fungal ecology and general ecology terminology have inherent problems, such as an incongruent combination of mechanisms and outcomes within a set of terms, making it difficult to compare interactions. Other ambiguities that need to be resolved, specifically, are those between descriptions of interactions involving nutrient acquisition and those of direct, nonnutrient-related interactions. Difficulties arise in application of general ecology terminology to fungal ecology because fungi are sedentary, indeterminate organisms. Some general ecology terms imply motility or net effects, such as death, that could be avoided by indeterminate growth. Consistently applied terms with broad enough definitions to apply across all fields within ecology would be most useful, though it is recognized that more exact characterization of interactions can be achieved by adding descriptors that are specific to a field of ecology to the broadly defined terms.

It is the goal of this chapter to introduce a new approach to categorization of ecological terminology and its application to fungal ecology based on terms used in classic and current literature. I begin with a history of interspecific interaction terminology used in general ecology and in fungal ecology. It is beyond the scope of this chapter to publish a comprehensive review of all current textbooks describing interspecific

Table 13.1 Possible Outcomes from Bilateral Interactions

	Effect of Species B on Species A		
Effect of A on B	**–**	**0**	**+**
–	–/– Synnecrosis	0/– Allolimy	+/– Parasitism
0	–/0 Amensalism	0/0 Neutrality	+/0 Commensalism
+	–/+ Predation	0/+ Allotrophy	+/+ Symbiosis

Adapted from Burkholder, *Am. Sci.*, 40, 601–631, 1952.

interaction terminology, though some have been reviewed previously (Abrams, 1987). Because terms have been applied differently in different fields, comparison of interactions across fields in ecology is difficult. It is intended for this chapter to clarify the sources of the inconsistencies, which are discussed in separate sections for general and fungal ecology, and to describe particular problems with application of general ecology terminology to fungi due to their growth form and life histories. I discuss temporal dynamics of interactions and measures best used to describe outcomes. I discriminate between interactions involving nutrient acquisition and those not involving nutrient acquisition and propose a revised set of terms. It is anticipated that this chapter will raise questions and generate discussions about the best use of interspecific interaction terms. For that reason, it is hoped that this paper will facilitate discussions among ecologists by categorizing terms according to mode of nutrient acquisition and by making terms that are currently in use commensurate.

13.2 GENERAL ECOLOGY TERMINOLOGY

Description of interspecific interactions began when deBary (1887) first described close relationships between two organisms as *symbioses*, regardless of positive or negative outcome of interaction. He divided these symbioses into *saprophytic* and *parasitic* interactions. These categorizations were based on mode of nutrient acquisition and were adequate descriptors because they were the only ones. Burkholder (1952) subsequently made progress by characterizing biological interactions according to outcomes (Table 13.1), which was useful because it is practical to measure a population's size in many instances. However, he initiated a long history of the confusing use of the terms *symbiosis* and *parasitism* by equating them with outcomes and by describing "symbiosis" as only a mutually beneficial interaction. Odum (1959) modified Burkholder's description by differentiating between the effects on organisms when they did and did not interact (Table 13.2), though this distinction is not always practical. Harper (1961), Schoener (1983), and Hodge and Arthur (1996) have all described alternate terms for antagonistic interactions, with Schoener dividing competition according to the particular mechanisms involved. In the more than a century since deBary's report of types of interactions, we have had many contributions to the pool of terms available to describe interspecific interactions, but little consistency across the sets of terms.

Table 13.2 Effects on Organisms When They Are and Are Not Interacting

	When Not Interacting		When Interacting		
Type of Interaction	**A**	**B**	**A**	**B**	**Result of Interaction**
Competition	0	0	–	–	
Amensalism	0	0	–	0	
Neutralism	0	0	0	0	
Commensalism	–	0	+	0	Obligatory for A
Protocooperation	0	0	+	+	Facultative for both
Mutualism	–	–	+	+	Obligatory for both
Parasitism/predation	–	0	+	–	Obligatory for A

Adapted from Odum, *Fundamentals of Ecology*, W.B. Saunders Co., Philadelphia, 1959.

13.3 FUNGAL ECOLOGY TERMINOLOGY

Mycologists have employed some terms previously used to describe interspecific interactions based on outcome (Cooke and Rayner, 1984; Rayner and Webber, 1984; Rayner and Boddy, 1988b; Zabel and Morrell, 1992). Not all terms that are available apply to fungi, and some definitions have to be changed slightly in order to be more accurate in their application to fungi. In addition, terms were developed by mycologists to describe specific mechanisms exhibited by fungi during interactions (Rayner and Boddy, 1988a, 1988b; Table 13.3) and mechanisms for competitive or antagonistic interactions in detail (Lockwood, 1992; Wicklow, 1992; Boddy, 2000), adding to the pool of available terms. Because mycologists have tried to use some available terms, but required additional descriptors for

Table 13.3 Descriptions of Bilateral Fungal Interactions

Interaction	**Outcome**
Neutralistic	0/0, 0/+, +/0
Mutualistic	+/+
Competitive	0/–, –/0, –/–
Primary resource capture	(Mechanism)
Combat	(Mechanism)
Defense (antagonism at a distance, hyphal interference, mycoparasitism, gross mycelial contact, deadlock)	(Mechanism)
Secondary resource capture (replacement)	(Mechanism)

Note: Various outcomes may occur for interactions that are considered a subdivision of competition. However, these terms actually describe the mechanism involved in the interaction.

Adapted from Rayner and Boddy, *Fungal Decomposition of Wood*, John Wiley & Sons, New York, 1988b, with information from Boddy, *FEMS Microbiol. Ecol.*, 31, 185–194, 2000, added.

specific types of fungi (e.g., wood decomposers), we now have a set of terms with slightly different meanings in fields of ecology, plus a combination of some terms describing outcomes and others describing mechanisms. Mycologists, in their effort to reduce the number of new terms by borrowing some from general ecology, yet requiring additional terms for specific circumstances particular to fungi, have compounded the confusion arising around a clear description of interspecific interactions.

13.4 DISCUSSION OF GENERAL ECOLOGY TERMINOLOGY

While Burkholder's (1952) characterization helped clarify ecological concepts, some of Burkholder's terms do not apply to fungi and some are redundant. The terms *allotrophy*, defined as feeding another organism, and *allolimy*, defined as starving another organism, appear to be associated with higher organisms than fungi because fungi cannot actively feed or starve other organisms. Burkholder used *symbiosis* to describe only mutualistic interactions, whereas deBary's original use of *symbiosis* referred to any close relationship between two or more organisms (deBary, 1887), whether harmful or beneficial. Burkholder used *synnecrosis* to refer to interactions that are negative for both organisms, implying that both organisms in a mutually antagonistic relationship will die; however, death is not inevitable. Burkholder's categorization of interactions according to outcome was useful, but some of his terms, such as *allotrophy*, *allolimy*, *symbiosis*, and *synnecrosis*, are not generally applicable, especially to fungi.

Odum's modification enabled ecologists to describe interactions as facultative or obligate. Odum also adopted some new terms for outcomes described by Burkholder and added some categories. According to Odum, the difference between *protocooperation* and *mutualism* is whether both organisms are not affected (0/0, protocooperation) or are negatively affected (–/–, mutualism) in the absence of the interaction. This distinction is difficult to assess because comparative measurements can only be made relative to the interaction. Both the null effect and any negative effect will appear negative relative to any positive effect of the interaction. Odum also replaced Burkholder's term *synnecrosis* with *competition*, which is equally misleading, because it describes only one possible mechanism leading to an outcome in which both organisms are inhibited (–/–) (see Abrams, 1987). Other mechanisms, such as allelopathy, may also result in mutual inhibition. Odum's use of *parasitism* and *predation* to describe outcomes that are positive for one organism and negative for the other (+/–) is also confusing. Parasitism and predation are modes of nutrient acquisition, or nutritive interactions, which should be considered separately from nonnutritive interspecific interactions (see below).

Harper (1961) used the term *interference* rather than *competition* to describe antagonistic interactions in plant ecology. He maintained that this term, rather than competition, could be applied more uniformly to all fields because of the many different connotations that exist within different fields concerning competition. Interference was later used in fungal ecology to describe a subdivision of competition, together with exploitation (see below, also Tinnin, 1972; Lockwood, 1992; Wicklow, 1992). Both interference (whether used as a synonym or as a subdivision of competition) and exploitation describe mechanisms involved in antagonistic behavior.

Schoener (1983) used a set of six terms to describe the mechanisms of competition: *consumptive*, *preemptive*, *overgrowth*, *chemical*, *territorial*, and *encounter*. His terms may be appropriate in some situations to describe antagonistic interactions, but they describe only mechanisms for one type of outcome. These mechanisms of antagonism should be treated separately from the outcomes.

Arthur and Mitchell (1989) used similar terms: *competition* (–/–), *amensalism* (–/0), *contramensalism* (+/–), *commensalism* (+/0), and *mutualism* (+/+). However, they do not distinguish mechanism from effect, nor nutritive from nonnutritive interactions. They suggested that mechanism should be separated from outcome, but their system is not structured to do so.

Problems in describing interspecific interactions also arise from variability in responses due to temporal, spatial, and environmental variability. In addition, for all of these schemes of interactions, more than one mechanism of interaction may be occurring simultaneously. Each of these schemes relies upon an average of outcomes to determine the net effect, and the length of time of observation may affect the observer's determination of type of interaction (Arthur and Mitchell, 1989). Interactions are likely to vary spatially and change over time (Hodge et al., 1999). These interactions are dynamic and are conditional upon the abiotic and biotic environment in which they occur (Bronstein, 1994). For instance, pH affected the ability of nematodes to antagonize root pathogenic fungi (El-Borai et al., 2002). *Aspergillus ochraceus* was most effective at antagonizing six other species of maize spoilage fungi under moderate water availability at 18°C (Lee and Magan, 1999, 2000). When water was freely available, *Alternaria alternata* and *Aspergillus niger* became dominant (Lee and Magan, 1999). At 30°C and moderate water availability, *A. ochraceus* was dominated by other fungi except *A. alternata* (Lee and Magan, 2000). So the outcome of the interaction between *A. ochraceus* and *A. alternata* is highly dependent upon water availability and temperature. Water potential also affected outcome of interactions between fungi colonizing ash twigs (Griffith and Boddy, 1991), and water activity and temperature affected interactions of fungi isolated from maize (Sanchis et al., 1997). Environmental factors affect parasites (Michalakis et al., 1992) and mycoparasitism (reviewed in Lumsden, 1992). Nutritional differences in the environment may also regulate outcomes. Nutritional or structural differences between agar and wood caused outcomes of interactions between fungi grown on wood discs to be different from interactions on nutrient agar (Holmer and Stenlid, 1993). Interactions between *Ceratocystis ulmi* and elm bark saprotrophs were different on malt extract agar (MEA) in petri dishes than in elm logs (Webber and Hedger, 1986) as well. However, outcomes of interactions between wood decay fungi plated against *Armillaria luteobubalina* on MEA did correspond to interactions in Eucalyptus wood (Pearce, 1990). For this reason, conditions in which observations of interactions are made should be made clear along with timing of "stage-specific phenomena" (Bronstein, 1994) to more fully understand the nature of the interaction.

In addition to abiotic environmental effects, biotic components can affect interactions between organisms. For example, *Dendroctonus* bark beetles do worse in the presence of phoretic *Tarsonemus* mites because *Ophiostoma* carried as a food source by the mites antagonizes the mycangial fungi of the bark beetle, reducing the food source of the bark beetle (Lombardero et al., 2003). This agonistic (–/+) interaction between the beetle and the mites is the result of indirect effects of the biotic environment, whereas the direct effect is a commensal (0/+) relationship between the mites and the beetles. Therefore, it should be made clear whether the results of an interaction are direct or indirect (see also Callaway and Walker, 1997). Tritrophic interactions ultimately can affect the outcome of interactions between organisms distantly related, such as the cereal aphid parasitoid (*Aphidius rhopalosiphi*) and an entomopathogenic fungus (*Erynia neoaphidis*), which utilize the same food source (the grain aphid, *Sitobion avenae*; Fuentes-Contreras et al., 1998). Timing of arrival of the parasitoid relative to fungal spore arrival will affect the outcome of the interaction. Similarly, single-genet isolates of *Marasmius androsaceus* were identified in younger plots, whereas discontinuous distribution was observed in older plots (Holmer and Stenlid, 1991). Successional changes in species distributions and abun-

dances likely affect the interactions between species within the environment (Connell and Slatyer, 1977). Spatial patterning of 60 taxa of mycophagous insects on 66 mushroom taxa were more closely related to aggregates of the insect species than to species of food source, indicating dispersal as the reason for existence on a particular fruit body, rather than niche differentiation by food type (Wertheim et al., 2000). Insects were cohabiting (0/0) the mushrooms rather than coantagonizing (–/–) one another and eliminating each other from the resource. Consequently, measures of biotic environmental conditions can be affected by the temporal and spatial scales in which measures are taken and can influence interpretation of outcomes. Size of population is known to affect outcomes of interactions (Holmer and Stenlid, 1993) as well as other factors inherent in measure of population growth rate. Therefore, type of measurement taken (growth, birth rate, death rate, reproductive success) can influence conclusions from interspecific interactions (Abrams, 1987). A comprehensive terminology should account for these variables.

13.4.1 Application of General Ecology Terminology to Fungi: Distinguishing Features of Fungi

Fungi are sedentary microscopic organisms with an indeterminate growth form. Although fungal hyphae are microscopic, population distribution and dispersal of spores often cover large geographic areas (e.g., see Smith et al., 1992). Likewise, the functions performed by fungi are often related to an ecosystem or landscape scale (e.g., see Kuyper and Bokeloh, 1994). Hence, interactions at the microscale may have broad effects. The indeterminate growth form allows fungi to interact with different organisms at different places across a mycelial network. Each interaction may simultaneously have different effects. This growth form allows an individual fungus to wall off an area in the event of antagonism (–/0), which may result in two individuals plus a senesced portion of mycelium. Hence, quantification of populations is difficult and populations may not be reduced as a result of antagonism.

Fungi, along with many other microorganisms, are not large enough to take in many molecules that would be valuable sources of carbon and nutrients, so they release extracellular compounds to decompose substances prior to uptake (Bruce et al., 1984; Ghisalberti and Sivasithamparam, 1991; Dandurand and Knudson, 1993; Score et al., 1997). Production and release of oxalic acid, known to aid in weathering of minerals and thereby increase nutrient availability, by the mycorrhizal fungus *Paxillus involutus* was affected by a form of nitrogen in media and the concentration of calcium and bicarbonate ions (Lapeyrie et al., 1987). Oxalic acid was also produced by brown-rot fungi, though not in the same quantities by all species (Espejo and Agosin, 1991). Oxalic acid reduced pH in brown-rot cultures (Dutton et al., 1993), consequently increasing favorable conditions for enzyme activity. In the study by Espejo and Agosin (1991), oxalic acid that was produced was then oxidized to CO_2 during the process of cellulose depolymerization. Release of oxalic acid by one fungus may benefit many others in the vicinity. Other extracellular substances released by fungi (whether enzymes or waste) could be used by other microorganisms as a nutrient source or may enhance or be inhibitory to their growth (Garbaye, 1991; Score et al., 1997).

These particular features make application of some of the previously used general ecology terminology for interspecific interactions to fungi not completely appropriate. For instance, Burkholder's use of *allotrophy* and *allolimy* do not apply to sedentary organisms, and his *synnecrosis* has an outcome (–/–) that might occur in hyphal tips in fungi but will not kill the organism, as implied by the term. Odum uses mechanisms for antagonism that confuse nutrient acquisition. This distinction is particularly important for microorganisms because nutrient acquisition occurs extracellularly, will nearly always impact other organ-

isms, and may not always do so in a negative manner (such as in the case where one organism releases extracellular enzymes to decompose a substrate and another organism benefits by using the enzymes or the products of the reaction as a nutrient source; Garbaye, 1991). Consequently, mycologists have adopted slightly different terms to describe antagonistic (–/0) interactions (exploitation and interference) that begin to differentiate nutritive from nonnutritive interactions but do not do so explicitly. General ecology terms should be selected and applied carefully to describe microbial interspecific interactions to ensure that the connotation is correct and that the meaning of a single term is not required to change among fields for proper application.

13.5 DISCUSSION OF FUNGAL ECOLOGY TERMINOLOGY

Rayner and Boddy (1988a, 1988b) and Boddy (2000) modified Burkholder's schema (Table 13.3), but included only competitive, neutralistic, and mutualistic interactions. These three main categories are similar to previous definitions in that they are based on outcomes of interactions. Their divisions within competition — primary resource capture, combat (antagonism at a distance, hyphal interference, mycoparasitism, or gross mycelial contact), defense, and secondary resource capture — however, pertain to the biology and action of the organisms and, thus, refer to mechanisms.

In this system, the subdivisions of competition are actually mechanisms of interactions with various outcomes, some of which are not the result of competition, and therefore should not be described as subdivisions of competition. *Primary resource capture* describes colonization of a resource by one organism but may not result in competition for resources (ruderals generally do not exhibit competition). There is no interaction in primary resource capture, so no outcome can be identified. *Combat* refers to "interference competition," according to Rayner and Webber (1984). This is particularly confusing because their subdivisions of combat (*defense*, defending a resource through interference; *secondary resource capture*, replacement by competing for resources and using interference) refer to both interference and resource competition. Additional confusion arises because *defense* only describes an interaction from the perspective of one organism. The outcome of the interaction depends on the effects of the second organism. For instance, fungus A may defend its territory through the production of allelochemicals. However, if fungus B is involved in secondary resource capture, and the allelochemicals produced by fungus A did not result in complete inhibition of fungus B, then fungus A would be harmed regardless of its defense mode (–/+) because fungus B would acquire resources from A. Alternatively, if both fungus A and fungus B were involved in defense only, the result might be a deadlock in which both organisms might be harmed (–/–) because they have expended resources. Although the attempt made by Rayner and Boddy to describe fungal interactions with underlying mechanisms is a much needed addition to fungal ecology, it is a very specific set of terms that are mainly applicable to wood decomposer fungi. The mixture of terms within their schema is cumbersome, and the descriptions of mechanisms are not broadly applicable to diverse organisms in other disciplines in ecology.

Within fungal ecology, *interference competition* has been used to describe indirect inhibition (e.g., allelopathy or antibiosis) (Lockwood, 1992; Wicklow, 1992). This contrasts with *exploitation competition* or *resource competition*, where organisms compete directly for a resource such as nutrients or space (Lockwood, 1992). While some argue that interference and exploitation do not accurately describe coantagonistic (–/–) fungal interactions (Cooke and Rayner, 1984; Boddy, 2000), any of these terms, along with the terms proposed by Rayner and Boddy that were discussed above, could be used to describe

Table 13.4 Distinction between Fungal Interactions Involving Nutrient Acquisition and Those That Do Not Involve Nutrient Acquisition

Nutritive Interactions (Interactions between a fungus and another organism from which the fungus receives nutrients)	**Nonnutritive Interactions (Interactions between a fungus and another organism from which the fungus does *not* receive nutrients)**
Biotrophism	Coantagonism
Necrotrophism	Antagonism
Saprotrophism	Agonism
	Cohabitation
	Commensalism
	Mutualism

Note: Each nutritive interaction may occur simultaneously with any nonnutritive interaction in one organism, but the interactions are distinct. Terms used for nonnutritive interactions are described in more detail in Table 13.5.

mechanisms of antagonistic or coantagonistic behavior. When mechanisms specific to a group of organisms are used in combination with general outcome-derived terms, a more complete description of the interspecific interaction (described further below) is possible.

13.6 PROPOSED CATEGORIZATION OF INTERSPECIFIC INTERACTIONS

Ecological terms describing interspecific interactions incorporate a mixture of mechanisms of interaction involving nutrient acquisition and not involving nutrient acquisition. For instance, interactions involving organisms that feed on other organisms directly should be separated from interactions in which organisms interact directly with one another for a pool of nutrients that is indirectly depleted as a result of the interaction. I propose that two types of interactions be considered: (1) interactions between an organism and any organism from which it directly receives nutrients, *nutritive interactions*, and (2) interactions between an organism and any organism from which it does not directly receive nutrients, *nonnutritive interactions* (Table 13.4). These interactions should be considered separately because the underlying mechanisms are different and are not directly comparable.

13.6.1 Nutritive Interactions

Nutritive interactions were described by deBary in 1887 as *saprophytic* and *parasitic*. Contemporary terms result from refinement of deBary's concepts to include *biotrophy* (obtaining nutrients from the living cells of the host, e.g., parasitism; Barak and Chet, 1986; Figure 13.1), *necrotrophy* (acquiring nutrients by killing an organism, e.g., predation), and *saprotrophy* (acquiring nutrients from dead material, e.g., decomposition) (Lewis, 1973; Luttrell, 1974; Cooke and Rayner, 1984; Douglas, 1994). DeBary's concept of classifying organisms, and fungi in particular, according to type of nutritive interaction persists in these schemes and in current use.

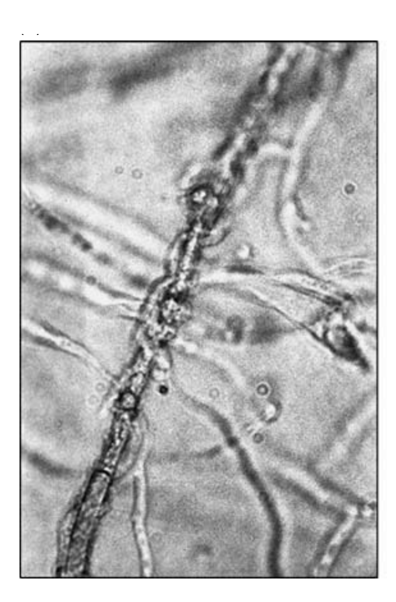


Figure 13.1 Example of potential biotrophic nutritive interactions. *Fomitopsis pinicola* (Swartz: Fr.) Karst encounter with *Cenococcum geophilum* Fr. on modified Melin-Norkrans medium (MMN) at 20°C.

DeBary originally suggested that the classes of nutrient acquisition that he proposed were not mutually exclusive categories, but overlapped, and this continuum has been suggested by others (Lewis, 1973; Harley and Smith, 1983). Under this classification, the often cited example of mycorrhizae (see also below) would be an example of bilateral biotrophism, not mutualism. This removes the frequently discussed problem of mycorrhizal interactions not always having a positive outcome for both the host and the symbiont. The cost and benefit to each organism are independent of the categorization of the interaction. Biotrophisms may be facultative or obligate. Organisms may alternate between nutritional modes throughout their lives (Luttrell, 1974; Cooke and Rayner, 1984; Douglas, 1994), or among stages in their life cycles. For example, an organism may be saprotrophic in one stage of its life cycle and become biotrophic in another (Bateman, 1978). The ability to adjust the mode of nutrient acquisition in response to changes in resource availability might allow organisms to avoid direct competition for a particular resource with another organism (Cooke and Rayner, 1984). Knowing the mode of nutrient acquisition of organisms can help to predict the types of nonnutritive interspecific interactions that may occur (e.g., two saprotrophs might be more likely to compete than would a saprotrophic and a necrotrophic fungus).

13.6.2 Nonnutritive Interspecific Interactions

Nonnutritive interspecific interactions are abundant in nature and important to population structure (Hairston et al., 1960). However, few nonnutritive fungal interactions have been documented. The few investigations reported have focused on competition (Rayner and Boddy, 1988b; Shearer and Zare-Maivan, 1988; Lockwood, 1992; Wicklow, 1992; Wardle et al., 1993; Shearer, 1995; Holmer and Stenlid, 1997a). Dodds (1997) attributes this bias toward reporting of competitive interactions to error in experimental design (but see Wardle et al., 1993; Hodge et al., 1999). Even when a null hypothesis of no interaction is tested statistically, as recommended by Dodds (1997), careful attention must still be paid to

experimental design. Attributing colonization of a substrate to only one organism (all or nothing) can lead to conclusions of antagonism (–/0), when cohabitation (0/0) of the substrate may have existed (Holmer et al., 1997).

13.6.3 Symbiosis

In the sense used by deBary, *symbiosis* refers to any close relationship, whether positive or negative, between two organisms, involving nutrient acquisition or not. Mycorrhizae would be symbiotic biotrophisms — close relationships involving nutrient acquisition. In mycorrhizae, fungi acquire carbon from and exchange nutrients with host plants (Allen, 1991; Smith and Read, 1997). Other examples of nutritive symbioses would include gut parasites extracting nutrients from hosts and necrotrophism of, or feeding on, mycangial fungi by bark beetles (Lombardero et al., 2003). These would also be biotrophic symbioses. Ant–membracid interactions would be mutualistic symbioses — close relationships with positive effects on the population size of both organisms involved, but not involving direct nutrient acquisition (Cushman and Whitham, 1989). The membracid nymphs receive protection from predators by the ants and the ants use a waste product of the membracids, not directly feeding on the membracids. Other examples of nonnutritive symbioses include a commensal symbiosis — coprophilous fungi that require passage of spores through an animal gut to germinate but neither contribute to nor extract nutrients from the host. Both nutritive and nonnutritive interactions can be symbiotic, following from deBary's (1887) definition of symbiosis, a close relationship between two organisms.

DeBary (1887) only used symbiosis to refer to modes of nutrient acquisition. Burkholder (1952) originated the description of interspecific interactions by outcome and used the term *symbiosis* more narrowly to describe a mutually beneficial outcome in interspecific interactions; this use continues to be popular in some countries. However, the common use of the term *mutualism* synonymously with *symbiosis* has led to the blurring of the nutritive and nonnutritive interactions. Because *symbiosis* encompasses both nutritive and nonnutritive interactions, careful attention must be made to distinguish among them when describing a symbiosis; otherwise, more descriptive information for these interactions may be lost. A description of the specific nature of symbioses can be more clear by adding nutritive or nonnutritive descriptors rather than using symbiosis synonymously with one type of nonnutritive interaction (mutualism). To aid future studies, the remainder of this discussion will focus on nonnutritive interspecific interactions because the terminology used to describe them requires further refinement, whereas terms used to describe nutrient acquisition interactions are adequate.

13.7 PROPOSED CLASSES OF NONNUTRITIVE INTERSPECIFIC INTERACTIONS

Outcomes of nonnutritive interspecific interactions are more readily compared across disciplines in ecology than are mechanisms, although both categories are necessary for full understanding of the interaction. Information regarding mechanism can be used to modify descriptions, but terms based on mechanism alone will not clarify terminology. Measures taken to evaluate outcome are dependent upon the purpose of the investigation (Figure 13.2). Definitions of nonnutritive interspecific interactions are given below with examples from fungal ecology.

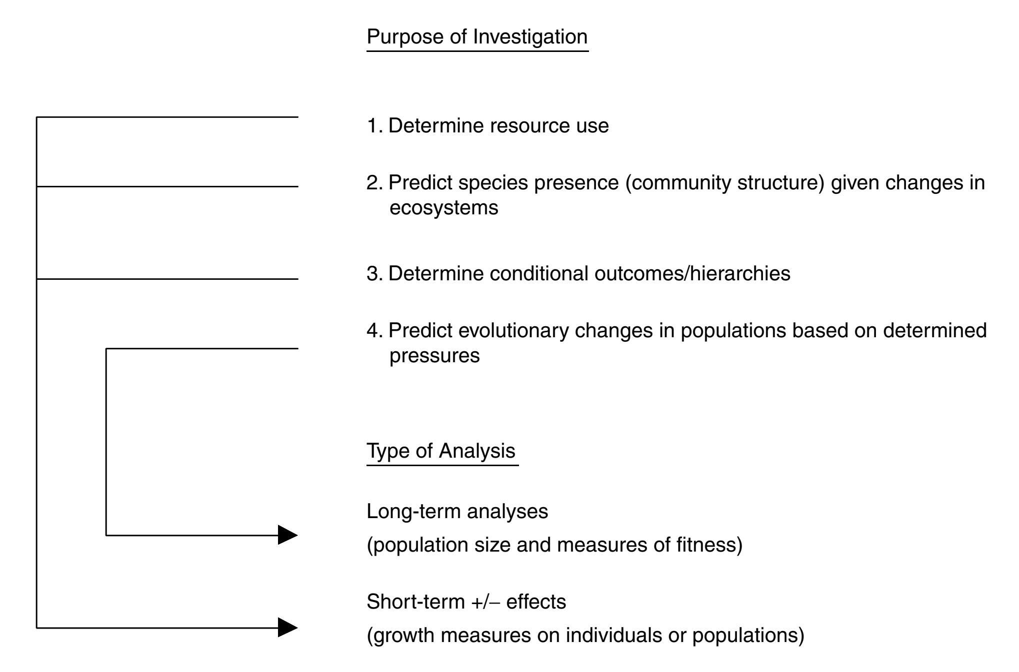


Figure 13.2 Key for determination of type of measures to take to determine outcomes of interspecific interactions.

13.7.1 Coantagonism

Coantagonism refers to any interaction that results in a negative outcome for both members (–/–; Figure 13.3a and Figure 13.4a). Mechanisms of antagonism may be different for either fungus, however, and the outcome may not be equally strongly negative for each fungus (Holmer and Stenlid, 1997a). It is the combination of effects, with mainly negative effects for each, that results in coantagonism. The term *coantagonism* is preferable to *competition* to describe bilateral mutually antagonistic interactions because *competition* describes only one mechanism of antagonistic interactions. *Coantagonism* is more consistent with terms currently used to describe other outcomes of interspecific interactions.

In studying fungal interactions in culture, three ways to identify antagonism follow. If fungus A is antagonized by fungus B, fungus A may respond by (1) asymmetrical inhibition only in the direction of B; (2) general decrease in overall colony size, irrespective of direction; or (3) increased radial colony diameter, but decreased hyphal diameter and mycelial density. The latter may not actually have a negative effect on the antagonized fungus. In this discussion, *coantagonism* refers to the joint action of the first two types of antagonism.

Examples of coantagonism are relatively abundant in the mycological literature. Nine species of brown-rot fungi coantagonized one another in plate pairings on malt extract agar (Owens et al., 1994), though some species combinations were likely involved in antagonism (–/0) rather than coantagonism (–/–). The same is true for seven species of white-rot fungi and for interactions between species of brown-rot and white-rot fungi. These interactions are further described by the mechanisms resulting in this outcome, replacement or deadlock (see Table 13.5 and Section 13.8). *Coniophora puteana* and *Scytalidium* spp. produced laccase (a widespread phenol-oxidizing enzyme that can cause mycelial morphogenesis) in the presence of one another (Score et al., 1997), as did *Serpula*

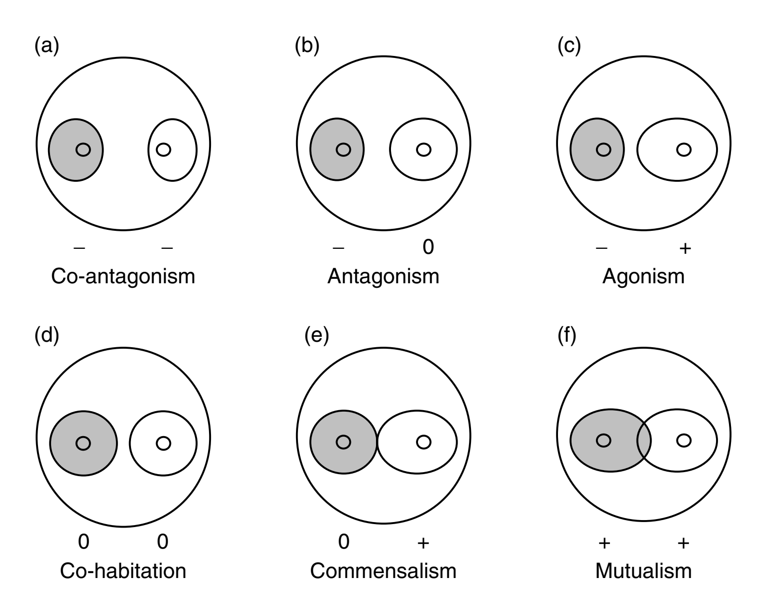


Figure 13.3 Possible interactions between two fungal cultures on petri dishes based on measurement of radial growth. Fungus A is the shaded colony and fungus B is the white colony.

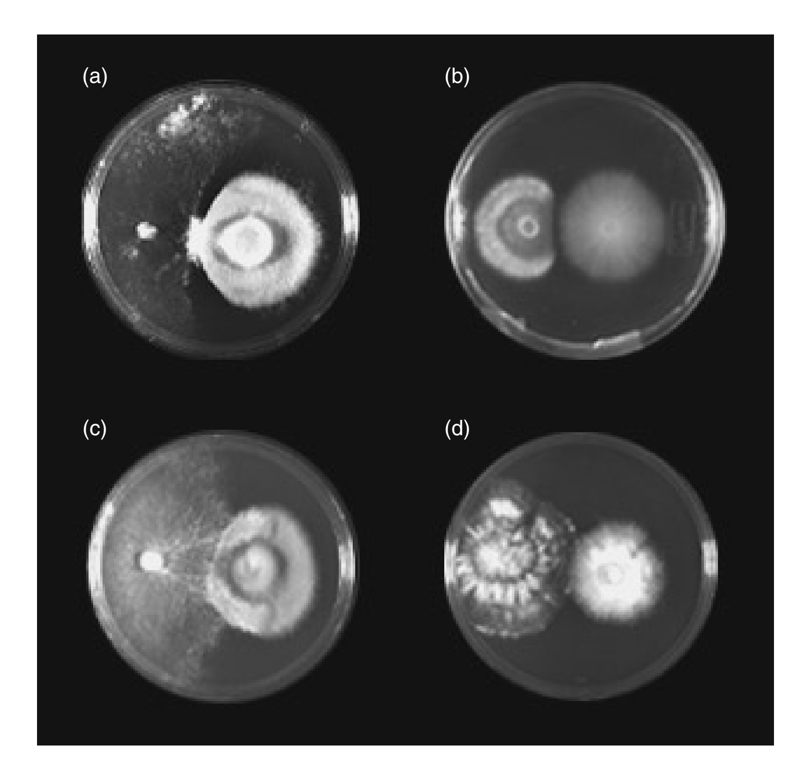


Figure 13.4 Examples of nonnutritive interspecific interactions. (a) Coantagonism (–/–) (*Trametes versicolor* (L.: Fr.) Pilat) + (*Paxillus involutus* (Fr.) Fr. on water agar at 20°C. (b) Antagonism (–/0) (*T. versicolor* (L.: Fr.) Pilat + *Laccaria bicolor* (Maire) Pat.) on water agar at 20°C. (c) Agonism (+/–) (*Fomitopsis pinicola* (Swartz: Fr.) Karst + *Paxillus involutus* (Fr.) Fr.) on MMN at 20°C. (d) Cohabitation (0/0) (*T. versicolor* (L.: Fr.) Pilat + *Thelephora americanus* Lloyd) on MMN at 20°C.

Table 13.5 Proposed Terminology for Nonnutritive Interspecific Interactions

Term	Outcome	Result/Effect	Examples of Mechanisms	Examples of Descriptors of Condition of Interaction
Coantagonism[a]	–/–	Death, decreased growth rate of both	Lysis, vacuolation, change in pH, interference (coallelopathy, coantibiosis), physical inhibition, nutritional competition (exploitation, primary resource capture), deadlock, defense	Temperature, pH, nutrient concentration, presence of other organisms, timing
Antagonism	–/0	Death, decreased growth rate of one	Lysis, vacuolation, change in pH, interference (allelopathy, antibiosis), physical inhibition	Temperature, pH, nutrient concentration, presence of other organisms, timing
Agonism	–/+	Decreased and increased growth rates	Interference (allelopathy, antibiosis)	Temperature, pH, nutrient concentration, presence of other organisms, timing
Cohabitation	0/0	Neutralism	Intermingling	Temperature, pH, nutrient concentration, presence of other organisms, timing
Commensalism	0/+	None, increased growth rate of one	Exudation, intermingling	Temperature, pH, nutrient concentration, presence of other organisms, timing
Mutualism	+/+	Increased growth rate of both	Exudation, intermingling	Temperature, pH, nutrient concentration, presence of other organisms, timing

[a] This term was originated in a Botany 542 class at Oregon State University.

lacrymans and three *Trichoderma* isolates. Tyrosinase (another phenoloxidase that is involved in synthesis of melanins) and peroxidase were also detected in pairings of the above species, indicating mechanisms of coantagonism. In whole-plant experiments, a coantagonistic interaction between root pathogenic nematodes (*Tylenchulus semipenetrans*) and root pathogenic fungi (*Phytophthora nicotianae*) was identified (El-Borai et al., 2002). The interaction resulted in lower population sizes of both organisms in the presence of the other than when alone, but only at low pH. When the pH was raised to favor the nematode, a one-way or antagonistic interaction occurred in which the nematode inhibited the fungus but was not inhibited by the fungus.

13.7.2 Antagonism

The above-described interactions could also occur unilaterally by one fungus inhibiting the growth of the other, while continuing to grow uninhibited itself (–/0; Figure 13.3b and Figure 13.4b). This is considered *antagonism*. The term *antagonism* is preferred to *amensalism* because it is consistent with *coantagonism*. *Competition* and *amensalism* have both been used in the past to describe interactions with a negative outcome for only one organism (–/0). If both *antagonism* and *coantagonism* are used, then it is clear whether both organisms or only one is negatively affected. As described above, fungus A may be inhibited by fungus B either in the direction of fungus B or uniformly in all directions. This could be the result of allelopathy ("antagonism at a distance," described by Boddy, 2000), such as production of phenol oxidases (Griffith et al., 1994; Yuen et al., 1999), other diffusible metabolites (Bruce et al., 1984; Bettucci and Silva, 1992), antibiotics (Lynch, 1990; Ghisalberti and Sivasithamparam, 1991), or physical exclusion of fungus A by fungus B ("hyphal interference" or "gross mycelial contact," described by Boddy, 2000).

13.7.3 Agonism

Agonism is an interaction in which one organism is harmed and the other benefits (–/+; Figure 13.3c and Figure 13.4c) (Lewis, 1985; Francis and Read, 1995). This term connotes a force opposing an antagonistic force and has been used previously in the literature, so it is recommended in place of Arthur and Mitchell's (1989) and Hodge and Arthur's (1996) *contramensalism*. While the terms *parasitism* and *predation* have frequently been used to describe this interaction, they should be restricted to descriptions involving nutrient acquisition, as described by deBary (1887).

It is possible that interactions between organisms exist that result in a negative outcome for one and a positive outcome for the other (–/+) and do not involve any type of nutrient acquisition. One should also note that the outcome for antagonism (–/0) is distinct from that for agonism (–/+) because the antagonistic fungus in antagonism does not benefit as it does in agonism. To illustrate, fungus A may inhibit fungus B by allelopathy, then grow in the direction of fungus B, to ultimately capture space formerly occupied by fungus B. Fungus A may benefit from changes that fungus B made in the substrate. In this case, fungus A is not receiving nutrients directly from fungus B, as in parasitism, but does benefit from fungus B. For example, Bettucci and Silva (1992) observed stimulated growth of *Laetiporus sulphureus* in the presence of extracts from *Trametes extenuata* and *Pycnoporus saguineus*, but inhibition of the two latter fungi by extracts of the former, resulting in (–/+) outcomes for each.

13.7.4 Cohabitation

Cohabitation is a more or less neutral interaction in which neither organism involved is significantly affected positively or negatively (0/0; Figure 13.3d and Figure 13.4d). However, because it is unlikely that two fungi could grow side by side without influencing one

another, an entirely neutral interaction is unlikely and, if it occurs, would probably be ephemeral. For this reason, the term *cohabitation* is proposed to replace *neutralism*. Neutralism implies the unlikely situation of absolute neutrality. Cohabitation, on the other hand, takes into account slightly positive or slightly negative interactions, but does not require absolute absence of effects. Cohabitation encompasses more of the continuum surrounding neutral interactions.

In field trials at three sites, inoculating stump roots with *Resinicium bicolor* did not appear to affect growth or occurrence of *Heterobasidion annosum* (Holmer and Stenlid, 1997b). Because more roots were infected with *R. bicolor* from naturally occurring strains than those that were inoculated with *R. bicolor*, it also appears that *H. annosum* may not affect *R. bicolor*. More *R. bicolor* was found in roots in the field plantation than had been inoculated, and more roots were infected naturally with *H. annosum* in the forest. Occasionally, root segments containing one fungus were more abundant than those containing the other, but both fungi were more abundant at different times in different locations, indicating a net neutral interaction, or a level of cohabitation. A study of pairings of soil fungi also reported cohabitation of soil fungal species and increased levels of total microbial biomass in mixtures over monocultures, perhaps indicating mutualism (Wardle et al., 1993). Interestingly, both studies indicated antagonistic or coantagonistic interactions in the title, not cohabitation or neutralism.

13.7.5 Commensalism

Commensalism is an interaction in which one organism is not affected and the other benefits (0/+; Figure 13.3E). Commensalism is well described in the literature, although there are few examples, particularly of fungi, in which this interaction is known to occur. It is likely, however, that along a continuum of interactions, fungus A may affect fungus B more positively, in which case this description may be fitting. Fungus A, because it is affected very little, could be considered not to have been affected. Bettucci and Silva (1992) report stimulated growth of *Laetiporus sulphureus* in the presence of extracts from *Panus tigrinus*, but no effect on *P. tigrinus* by extracts from *L. sulphureus*, resulting in a (+/0). Examples of stream invertebrates comminuting leaf litter and thereby benefiting downstream fungi that chemically decompose the organic matter, resulting in commensal interactions (+/0), are reviewed by Heard (1994). Another possible example of commensalism is when a large thallus may provide favorable conditions for a spore to germinate. The thallus would be able to affect the spore positively, but it is unlikely that the germinating spore would have much effect on the large thallus. Thus, magnitude of interaction may also be important in determining type of interaction.

13.7.6 Mutualism

Mutualism is an often overlooked interaction in interspecific fungal ecology in which two organisms both benefit (+/+; Figure 13.3F). Mycelia of two different species of fungi may be intermingling and yet may not be inhibited. Yet, if an interaction does not result in a distinct negative effect, it is often not considered. Many fungal interactions are the result of release of extracellular enzymes (Score et al., 1997), and it is unlikely that two fungal hyphae could grow side by side without affecting one another, and it is also unlikely that all effects are negative (Dodds, 1997). It is then necessary to investigate exactly what benefits one fungus might be acquiring from the other. Examples of mutualistic interactions between two fungi are not common in the literature, although they certainly must abound in nature due to the abundance of extracellular enzymes and secondary metabolites that are released by fungi (Frankland et al., 1982; Garbaye, 1991), which could be used directly as sources of nutrients by other fungi or indirectly if the by-products of the interaction

are of nutritional use or inhibit growth of competitors common to both fungi. Garbaye (1991) describes a number of interactions between mycorrhizosphere microbes, among them bacteria and actinomycetes producing citric acid, vitamins, and other growth factors such as indoleacetic acid (IAA) (Garbaye, 1994) that increase the growth of mycorrhizal fungi such as *Hebeloma crustuliniform*. Free-living nitrogen-fixing bacteria may also contribute ammonium to the fungus. Likewise, *Laccaria laccata* and *Laccaria bicolor* were increased in growth in the presence of four isolates of helper bacteria (Garbaye, 1994). The bacteria likely utilize carbohydrates and amino acids leaked by the fungus (Garbaye, 1991), completing the two-way, beneficial, but nonnutritive interaction.

13.8 RELATION OF PROPOSED TERMS TO GENERAL ECOLOGY

The set of terms proposed herein (Table 13.5) is a combination of previously used terms and new terms. Several of these terms originated in general ecology and should already be applicable to many disciplines in ecology. New terms such as *coantagonism* and *cohabitation* will need to be tested for their suitability in general ecology, and revisions will need to be made where necessary. These terms are based on outcomes of interactions that may be observed in diverse organisms. Mechanisms that may be specific to particular systems are given as examples of potential descriptors that might be used with each outcome. A single interaction can be most thoroughly described by including all information in numbered items, as read across a single row in Table 13.5. Because Connell and Slatyer (1977) have already given terms that are useful to describe longer-term interactions between community types (facilitation, inhibition, and tolerance), the proposed terms will be used for a relatively shorter period and for interactions between two species. Nonetheless, it will be important to note the period in which the observation took place and the life stage of the organism (column 5 in Table 13.5). It is also important to indicate how the interaction changes through time (what all the possible outcomes might be). It has become clear that interspecific interactions are conditional upon many environmental variables (Bronstein, 1994), and therefore, conditions of the interaction should become part of the description (column 5 in Table 13.5).

The new ideas presented in this paper are:

1. A concise review and comparison of previous terms used to describe interspecific interactions
2. Separation of descriptions of interactions according to nutritive and nonnutritive interactions
3. Recognition of difficulty in comparing outcome-based and mechanism-based terms
4. Clarification of a system that can incorporate both outcomes and mechanisms
5. Inclusion of fungi and generalization of a system for description of interactions for all taxa

This combined description with emphasis on outcome allows a much needed comparison of interactions between fields in ecology by equalizing terminology, along with identification of different mechanisms resulting in given outcomes within a field in ecology.

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Section 2

Function of Fungal Communities

14

Fungal Activity as Determined by Microscale Methods with Special Emphasis on Interactions with Heavy Metals

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14.1 INTRODUCTION

The fungi constitute a large, heterogeneous, and ubiquitous group of organisms characterized by a filamentous, multinuclear mycelium, which forms a system effectively exploiting the resources of the substratum. The walls of the hyphae, remaining in direct contact with the substratum, exert an important function in a free-living fungus. They contain a set of enzymes able to degrade extracellular nutrients, making them available for absorption. They may produce organic acids, which can take part in the transformation of insoluble salts of needed elements. The narrow, filamentous nature of fungi allows them to exploit tiny soil pores and the surface of soil aggregates not accessible for plant root hairs. Hyphae are also important for long-distance transport of absorbed elements, especially when symbiotically associated to plants as in mycorrhizas or forming lichens with algae or cyanobacteria.

Some fungi can have a strong metal-binding ability, resulting in altered biomass production and metabolic activities of other biota, both in cultures and in natural environments. These fungi may serve as biosorbents used for extracting and concentrating metallic elements from the liquid environment of industrial effluents. A whole family of biosorbent

compounds originating from fungal biomass, or substances synthesized on the basis of the structure of the fungal substances, was developed.

Metallic elements of specific gravity close to 5 or above are termed heavy metals. Despite their general feature of being potentially toxic to living biota, certain ones belong to trace elements essential for growth and metabolism, while others have no known function (Woolhouse, 1983). The tolerance to a given toxicity level, the mechanisms of adaptation, and the ability to sequester and accumulate metals from the external environment vary not only among different strains and species of fungi, but also at different stages of fungal development. Reliable knowledge on the developmental and physiological state of the investigated material will improve our understanding of the mechanisms involved in fungal interaction with metals.

The knowledge of the above exampled subjects is based on the use of different techniques. Atomic absorption spectroscopy (AAS), inductively coupled plasma–atomic emission spectrometry (ICP-AES), and nuclear magnetic resonance (NMR) spectroscopy were used to estimate the element content in the mycelium and to characterize the general response of a given species or strain to heavy metals. Microanalytical tools related to optical or electron microscopy have significantly enhanced our understanding of the interactions with the environment. The localization of elements on the cellular and subcellular levels using one of the microanalytical systems gives important data on element distribution and, by this, on the role of elements in physiological processes, interactions between elements, and reasons for their deficiency or toxicity. It also provides the link between physiological and anatomical studies, which is especially important when studying responses of organisms to environmental stress, such as the presence of naturally occurring heavy metals or metals introduced by pollution.

This chapter discusses the use of microanalytical tools to study microscale interactions of fungi with soil or other diverse substrata and with plants. It covers the diversity of techniques and the criteria that must be met for successful application of the methods.

14.2 CELLULAR AND SUBCELLULAR IDENTIFICATION, LOCALIZATION, AND MAPPING OF ELEMENTS

The distribution of elements in biological specimens on the cellular and subcellular levels may be determined by several methods. Detection of characteristic x-rays generated during the interaction of electrons with distinctive elements ($Z > 9$ mostly) in a specimen is an extension of the capabilities of a scanning or transmission electron microscope (SEM or TEM). X-rays are more often detected in energy-dispersive (EDS) than in wavelength-dispersive (WDS) mode. Electron energy loss spectroscopy (EELS) and electron spectroscopic imaging (ESI) is another technique exploiting the interactions with the inner shell electrons of distinctive elements ($Z = 3$ to 92). New insight into interactions between elements, interatomic distances, bond angles, and types and numbers of neighboring atoms can be gained when extended x-ray absorption fine-structure spectroscopy (EXAFS) or near-edge fine-structure electron energy loss spectroscopy (ELNES) is applied (Teo, 1986; Koningsberger and Prins, 1988; Williams and Carter, 1996).

The use of focused protons instead of electrons for the generation of characteristic x-rays is the basis of particle-induced x-ray emission (PIXE). Quantitative PIXE analysis benefits from the possibility of simultaneous use of proton backscattering (BS) or scanning transmission ion microscopy (STIM) techniques (Johansson et al., 1995; Mesjasz-Przybylowicz and Przybylowicz, 2002). These complementary techniques are used for accurate

determination of local changes of the specimen matrix (composition and thickness/areal density). In secondary ion mass spectrometry (SIMS) a specimen is eroded with the use of low-energy ions. In addition to electron and proton microscopy, the microprobe analysis might also be carried out with the laser microprobe mass analyzer (LAMMA) fitted to a laser light microscope with a high-energy pulse laser (Kuhn et al., 1995). Each method has its advantages and limitations. The differences involve the spatial resolution, detection limit, elements that can be detected, and access to qualitative and quantitative standards. Detailed information is given by Hall and Gupta (1984), Benninghoven et al. (1987), Kottke (1994), Johansson et al. (1995), Egerton (1996), Williams and Carter (1996), Bücking et al. (1998), van Steveninck and van Steveninck (1991), Leapman and Rizzo (1999), and Kuhn et al. (2000). A general advantage of microscale methods is the low amount of material necessary for the analysis and the possibility to control the physiological and developmental states on the ultrastructural level.

14.3 PREPARATION OF SAMPLES FOR ELEMENT ANALYSIS

A critical point of using microanalytical tools on biological material is the preparation of the samples, which should be properly cleaned and washed in ice-cooled water to avoid losses of elements such as K. The material should be lyophilized or chemically fixed as soon as possible. Drying/rehydrating and freezing/thawing of soil samples containing fungal hyphae might result in large decreases of metal concentration in the hyphae, in comparison from moist samples transported directly from the field and analyzed without delay (Berthelsen et al., 2001). However, drying and rehydrating occur frequently under natural conditions; therefore, results obtained on both dry and wet material are of biological interest.

Fungal ultrastructure is by now well documented. Artifacts resulting from insufficient preparation can be recognized by TEM. Changes are observed mainly in senescent and dead cells. Distinguishing between fixation-induced and natural changes usually requires experience. Methods accompanying the microanalytical tools are vital for the evaluation of the element localization. Without the guidance of some reliable microscopical observations, misleading interpretations can be made. Primary observations should be carried out on living mycelia of interest under a light microscope, often underestimated by modern researchers. A skillful scientist will be able to observe, e.g., cisternae 200 to 300 Å thick, far below the expected resolution of light optics, simply using phase contrast (Girbardt, 1965). The Nomarski contrast is another tool that is very useful in such research. The observations should be supported by data obtained from physiological studies.

Among available preparation techniques the best option is cryofixation. When working at lower magnifications of the SEM or proton microscope, the best solution is to cryofix the sample by plunge cooling or cryopunching, followed by freeze drying. A more sophisticated method is high-pressure cryofixation followed by freeze substitution. The most adequate protocol to study element distribution in cell walls seems to be anhydrous freeze substitution (Orlovich and Ashford, 1995). Cryosectioning of such material is, however, extremely difficult, and embedding in nonpolar resins seems to be a better option to obtain dry-cut sections of material for elemental analysis (Fritz, 1989). Ultrathin sections of 30 to 40 nm, as required for EELS microanalysis, are even more difficult to obtain, and wet sectioning is necessary. In the latter case, only the distribution of precipitated elements can be studied.

14.4 MICROANALYTICAL STUDIES ON STRUCTURAL AND BIOCHEMICAL DIFFERENTIATION DURING MORPHOGENESIS OF FUNGI AND FUNGAL–PLANT INTERACTION

The development of electron microscopy techniques resulted in detailed descriptive characterization of the ultrastructure of many groups of fungi and showed the diversity of this group of organisms. It is a challenge to resolve the significance of ultrastructural patterns, which would lead to understanding the role of particular elements in physiological processes, nutrient requirement, and deficiency. Qualitative and quantitative elemental analysis using microanalytical techniques can constitute an important step in understanding fungal differentiation and morphogenesis, at least by drawing our attention to particular events. PIXE has been used to determine the distribution and element concentration of macroelements such as K, P, S. and trace metals like Cu, Mn, Fe, and Zn in germ tubes of *Aureobasidium pullulans* and *Ophiostoma ulmi* (Brunton et al., 1988; Gadd et al., 1988). The highest concentrations of K, P, Na, and Mg were shown at the tip and in older parts of the mycelium, where new branches of the hyphae or the yeast-like cells develop. A different pattern of element distribution was found in the mycelium of *Aspergillus niger*, with the concentration gradient decreasing from the hyphal tip toward the older regions. Combined with the use of fluorescent indicator dyes distinguishing biologically available ions from the bound pool, this method may enrich our knowledge (Gadd et al., 1988). The accumulation of nitrogen-containing compounds within vacuoles of *Cenococcum geophilum–Pinus sylvestris* has been measured by use of EELS and ESI (Kottke et al., 1995a). In the hyphal sheath of *Amanita muscaria* mycorrhizas cultivated at two different atmospheric CO_2 concentrations and two different levels of nitrogen, the interaction between storage of glycogen in the cytosol and nitrogen compounds in the fungal vacuoles was shown (Turnau et al., 2001a).

Differences in element composition have been found between elongated and spherical bodies associated with septae of ascomycetes. P, N, and S are the most common elements of spherical bodies of *Sarcosphaera crassa*, while Ca is dominant in elongated bodies of *Disciotis venosa* (Turnau et al., 1993a). Different types of septal bodies can be present in the same species — elongated bodies within ascogenic hyphae and spherical bodies within vegetative mycelium (Turnau, unpublished material).

Long-distance transport has been studied using lanthanum (La) or cerium (Ce) as tracers and x-ray microanalysis or EELS/ESI to map the element distribution at the ultrastructural level in mycorrhizas. Results show that lanthanum is primarily transported apoplastically in hyphal and plant cell walls, arrested only by the Casparian band. It is occasionally taken up by the plant cells via endocytosis (Pargney and Le Disquet, 1994; Kottke et al., 1995b; Carnero-Diaz Le Disquet, 1996; Vesk et al., 2000). High-resolution ESI reveals the presence of La and Ce in the fungal vacuoles and its passage through the fungal porus (Kottke, 1991; Carnero-Diaz Le Disquet, 1996). The latter results obtained from chemically fixed material were supported by studies on material prepared by high-pressure freezing using Cs and Sr as tracers (Frey et al., 1997). It is obvious that plants and fungi interact in different ways with metals.

Microanalytical tools can also be useful to study infection processes by fungi and the reaction of plants to pathogens. PIXE reveals a depletion of K in regions of *Pisum* leaf infection by *Erysiphe pisi*. At the same time, an increase of Ca content has been observed in thickened walls around infected cells (Watt and Grime, 1988). The accumulation of Ni, Zn, Cu, Mn, Fe, Ca, Ti, As, and Sr accompanied by a drastic depletion of P, S, and K has been demonstrated in leaf areas of a resistant genotype of *Lagenaria sphaerica* (Cucurbitaceae) infected by a foliar pathogen, the powdery mildew *Sphaerotheca fuliginea* (Weiers-

bye-Witkowski et al., 1997; Mesjasz-Przybylowicz, 2001). The metal content increased within the first 4 days after infection, and actually, the data obtained were the first to show the concentration of heavy metals that can be reached within plant cells in response to a pathogenic fungus. The infected cells were obviously killed by the toxicity of the metals, followed by the deposition of Si reaching 23% of dry weight within necrotic lesions, suggesting the formation of a barrier protecting the surrounding tissues from the fungus.

14.5 TRANSFORMATION OF SOIL MINERALS BY FUNGI

The involvement of fungi in rock weathering and soil formation has been known for a long time. Especially lichen-forming fungi have received much attention (Schatz, 1963; Seyers and Iskandar, 1974). The weathering phenomena occurring at the lichen/basalt and granite rock interface have been studied with SEM accompanied by an EDS analyzer (Jones et al., 1980; Ascaso et al., 1995). Extensive etching and degradation of minerals due to the production of oxalic acid have been observed. Similar changes have been found when *Aspergillus niger* was cultivated in liquid growth medium supplemented with labradorite and clay material separated from basalt (Jones et al., 1980). This "mining" ability has been suggested as well for mycorrhizal fungi (Jongmans et al., 1997; van Breemen et al., 2000).

The production of oxalate crystals occurs in a wide range of ectomycorrhizal fungi (Graustein et al., 1977; Cromack et al., 1979; Paris et al., 1995; Unestam and Sun, 1995; Arocena et al., 2001) and has been suggested to play various roles, such as avoiding calcium and oxalate toxicity (Snetselaar and Whitney, 1990) and providing a hydrophobic coating that prevents hyphae from becoming hydrated, which could result in reduced microbial attack (Whitney and Arnott, 1987; Arocena et al., 2001). Encrustation of the mycelium with oxalate crystals may also serve as protection against grazing soil animals. Finally, oxalate crystals may play a role in water regulation, as has been suggested in the case of lichen-forming fungi (Clark et al., 2001). TEM/EDS analysis accompanied by several other techniques, such as NMR spectroscopy, and gas-liquid chromatography–mass spectrometry, has been used in an elegant study of exudation–reabsorption in a mycorrhizal fungus *Suillus bovinus* (Sun et al., 1999). X-rays were used to identify the released ions within the fluid droplets exudated on the surface of hydrophobic mycelium. The interaction of rhizomorphs with minerals has been studied using PIXE by Wallander et al. (2002). Ca originating from apatite, the least soluble calcium phosphate from aerated soils, has been shown on the surface of rhizomorphs in the form of calcium oxalate crystals. Some rhizomorphs are rich in K, which suggests that these fungi might be good accumulators of these elements and, as claimed by the authors, might play an important role in transferring K to trees. Oxalate crystals have also been occasionally reported on the mycelium and spores of arbuscular mycorrhizal (AM) fungi (Boyetschko and Tewari, 1986; Jurinak et al., 1986), but their presence has not been confirmed in subsequent studies carried out in pot cultures (Knight et al., 1992) and in field studies performed on soil samples from diverse vegetation types of the arid zone of California (Allen et al., 1996). These studies do not exclude oxalate formation in other species of AM fungi or under conditions other than those studied so far. Such studies are not easy without techniques involving scanning electron microscopy accompanied by microanalytical tools. As AM fungi cannot be grown separately in aseptic conditions, the production of oxalate by the plant cannot be excluded. It is also important to confirm the oxalate nature of the crystals. The data should be verified with other techniques such as ELNES (Lichtenberger and Neuman, 1997), as the presence of a strong Ca peak does not exclude other chelating agents.

Fungi also form cation-binding polyphosphate, stored in vacuoles. Al–polyphosphate complexes have been demonstrated by *in vivo* NMR spectroscopy of *Laccaria laccata* (Martin et al., 1994). EELS enables precise localization of the position of elements in the small, osmophilic vacuolar bodies of *Laccaria amethystea* mycelium cultured in the same conditions as *L. laccata* (Kottke and Martin, 1994). Al has also been found within the fungal wall (Turnau et al., 1994a, 1994b), often bound by phosphate-containing pigments. Although the galactosamine polymer of the cell wall has been implicated as offering a potential site for binding polyphosphates (Harold, 1962; Marschner, 1997), the description of Al localization would suggest pigments to be binding sites for Al (Turnau et al., 1994a, 1994b) rather than polyphosphates, as suggested by Väre (1990) and Tam (1995).

14.6 TRANSFORMATION AND COMPARTMENTALIZATION OF HEAVY METALS WITHIN THE FUNGAL MYCELIUM

Many fungi can adapt to growth in polluted sites. However, the mechanisms of resistance or tolerance to heavy metals are still rather fragmentarily elucidated. Saprobic fungi are particularly interesting, as they grow relatively easily in laboratory cultures and can be used as biosorbents for metals, e.g., in aqueous waste stream (Galun et al., 1983; Gadd and White, 1989; Huang et al., 1990). The first step of research involves the assessment of the ability of the selected fungus to accumulate the metals. Such data are usually obtained with atomic absorption spectrophotometry, but this gives no information on the distribution or compartmentalization of elements within the fungal hyphae or on the surface of the fungal wall. Microanalysis is important to explain the mechanisms of action, and in recognition of conditions under which the metals are not efficiently chelated, they may become cytotoxic.

14.6.1 Extracellular Sequestration of Heavy Metals

Elemental microbeam investigations indicated that heavy metals may be chelated on the surface or within the cell wall, or intracellularly, within the cytoplasm or the vacuoles. However, the distribution varies strongly among fungal strains and species, and among individual metal ions. Relatively much is known on substances such as organic acids that are exudated by the fungi and sequestrate heavy metals (Figure 14.1). The ability of *Aspergillus niger* to produce various organic acids that mobilize inorganic phosphates resulting in immobilization of Co and Zn has been demonstrated. The identification of the substances present in culture media has been carried out by various techniques, such as differential pulse polarography, ion chromatography, and gas chromatography–mass spectrometry. X-ray microprobe analysis has been very useful as a complementary tool that clearly demonstrates the deposition of crystals containing the different elements (Sayer and Gadd, 2001).

The activity of fungi in heavy metal-rich soils leads to the transformation of metals and metalloids by processes such as oxidation, reduction, and methylation, resulting in changed mobility and toxicity of heavy metals toward other living organisms (Gadd, 1993, 1996; Morley and Gadd, 1995; White et al., 1995; Morley et al., 1996; Jacobs et al., 2002). This process can also play a role in the leaching of contaminants from the soil and polluting of the groundwater. Microanalysis can be efficient in evaluation and development of bioremediation techniques. Pyromorphite ($Pb_5(PO_4)_3Cl$), which was believed to be a safe and stable mineral in urban and polluted soils, has been demonstrated to be susceptible to transformation by fungal phosphatases and to the activity of organic acids. This resulted

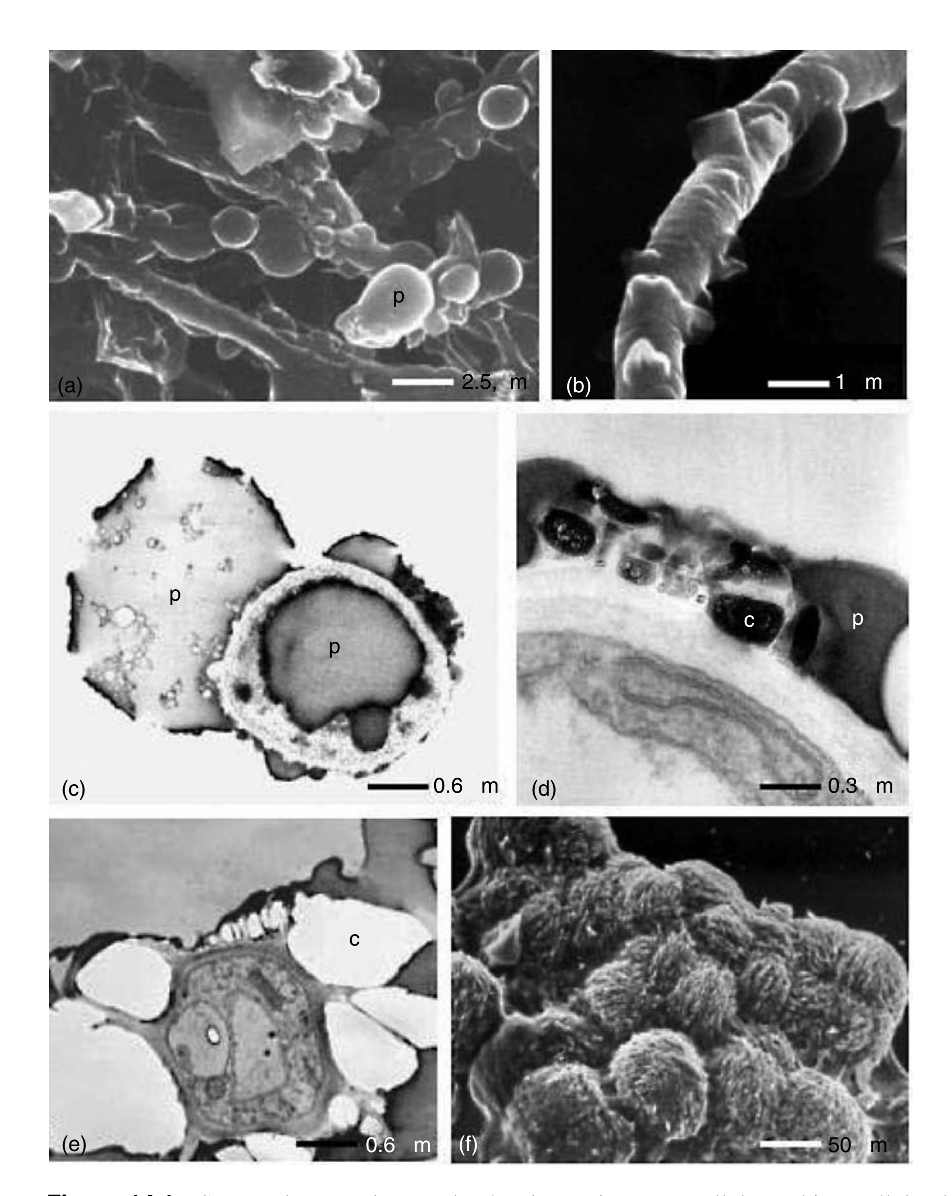


Figure 14.1 SEM and TEM micrographs showing various extracellular and intracellular depositions where heavy metals were detected. (a) Pigments excreted on the surface of mycelium. (b) Crystals produced on the mycelium. (c) Pigment material visible on the surface and within the mycelium. (d) Production of crystals underneath the pigment layer. (e) Disappearance of crystals after treatment with uranyl acetate. (f) Crystaloid structure of the mycelium wall of *Acremonium* sp. cultivated on medium with excess Cu. p, pigments; c, crystals. (Most images from Turnau et al., *Acta Soc. Bot. Pol.*, 71, 253–261, 2002. With permission.)

in significant alteration of mobility, toxicity, and transfer of Pb into other organisms (Sayer et al., 1999).

14.6.2 Binding Properties of the Hyphal Walls

The heavy metal-binding properties of fungal cell walls rely on the presence of the carboxyl, hydroxyl, and amino groups of the cell wall carbohydrates, proteins, and chitin/chitosan (Zimmermann and Wolf, 2002). The distribution of metals within cell walls has often been demonstrated by microanalytical tools and confirmed by other techniques, e.g., by analyzing cellular subfractions (Ono et al., 1988; Brady and Duncan, 1994a, 1994b; Yazgan and Özcengiz, 1994, Krantz-Rülcker et al., 1995; Gorovoi and Kosyakov, 1996; Zhou, 1999). In some cases, the metals are uniformly spread within the cell walls, which have been found to be the main storage pool of Cd and Zn (Blaudez et al., 2000; Frey et al., 2000). In other cases, they are localized in special structures. Volesky and May-Phillips (1995) found that uranium was precipitated within the cell walls in the form of fine, needle-shaped crystals. Research on the sorption of heavy metals by *Aspergillus niger* and *Mucor rouxii* has been carried out mainly with inductively coupled argon plasma spectrometry and complemented by a demonstration of Ag precipitations on the cell walls as colloidal silver using TEM/EDS (Mullen et al., 1992). Such studies can be further elaborated by the use of EXAFS, as has been done in the case of *Penicillium chrysogenum* (Sarret et al., 1998, 1999). In this work, Pb was shown to be bound to carboxyl and phosphoryl groups within the cell wall. The carboxyl groups had a high affinity for Pb, but were present in low amounts, while the phosphoryl groups of lower affinity were more abundant.

SEM/EDS microanalysis is valuable to understand why fungi such as *Armillaria* spp. can occur in extremely polluted places, although high concentrations of heavy metals can be toxic to mycelia in culture. Abundant fruit bodies and rhizomorphs have been noted on experimental plots treated with up to 5000 tons ha^{-1} of cadmium dust (Turnau, 1990). Both fruit bodies and rhizomorphs were often found in direct contact with pure industrial dust. Using EDS, Rizzo et al. (1992) found elements such as Al, Zn, Fe, Pb, and Cu located only on the outer, melanized parts of the rhizomorphs, but not in the interior. The accumulation of toxic levels of heavy metals may also serve as protection from antagonistic microorganisms in both polluted and nonpolluted environments.

Some fungi may become bluish while grown in the presence of excess Cu. In most cases, abundant production of oxalate is responsible for this alteration. However, this was not the case of *Acremonium pinkertoniae* (Figure 14.1). Observations carried out with SEM revealed crystals occurring within the cell walls. Differences in shape, localization, and effectiveness of Cu binding within the crystals led to further research with infrared spectroscopy, suggesting the involvement of cell wall components such as chitin (Zapotoczny, unpublished data). This information may be useful for the development of new soil-cleaning technologies.

During studies on compartmentalization of heavy metals within the mycelium, it is extremely important to control precisely the vitality of the fungal hyphae because when the metabolic activity is inhibited or absent, the accumulation of metal is mostly due to biosorption, resulting in its binding on the surface or within the cell wall (Tobin et al., 1984; Avery and Tobin, 1992). In addition, if the metal-to-biomass ratio is below 100 nmol g^{-1}, then metal accumulation is almost entirely dependent on biosorption of metal ions to the cell wall (Brady and Duncan, 1994a).

14.6.3 Metal Chelation within Cytosol and Vacuoles

Heavy metals that have entered the cytosol of the hyphae due to energy-dependent cellular transporters are immobilized by chelators such as phytochelatins and metallothioneins

(Lerch, 1980; Munger and Lerch, 1985; Howe et al., 1997). The nature of these compounds was studied in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida glabrata*, *Kluyveromyces marxianus*, and *Neurospora crassa* (Wemmie et al., 1994; Yazgan and Özcengiz, 1994; Li et al., 1997; Sajani and Mohan, 1998). Microscopic observations are useful to study this subject. The method adopted by Morselt et al. (1986) is the one most often cited. There are, however, several limitations to this technique; because no visible reaction occurs in dark pigments producing fungi, change of staining within the cell wall or a positive reaction in control samples was observed. In the study of extraradical mycelium of several mycorrhizas collected from heavy metal-rich sites, the positive staining reaction was shown only in *Hebeloma* spp. that have been found to act as bioexcluders of heavy metals. No reaction was observed in the case of *Rhizopogon roseolus* and *Suillus luteus.* The latter was shown to produce a positive reaction to the Gomori–Swift technique that visualizes S-S groups in proteins at the ultrastructural level. In the same fungi, the distribution of heavy metals was studied by EELS/TEM, revealing the presence of the heavy metals within the cytoplasm and vacuoles (Turnau, unpublished results). This might suggest that the staining according to Morselt et al. (1986) is specific for few substances.

Heavy metals that are taken up in the cytosol are subsequently transported into the vacuoles. Yeast tonoplast-located transporters are already known (Li et al., 1997; Klionsky, 1998). The presence of heavy metals such as Cd, Zn, and Cu within vacuoles has been originally indicated using microanalytical techniques in chemically fixed fungal material (Turnau et al., 1993b, 1993c). In freeze-dried mycelium studied with PIXE, these elements are usually nonuniformly distributed, and the elemental maps suggest the involvement of intracellular granular material in heavy metal sequestration (Figure 14.2 and Figure 14.3). Mycelia of *Fusarium* sp. and *Penicillium citrinum* have been found by means of TEM/EDS microanalysis to transform tellurium, leading to possible destruction of semiconductor thermoelectric cells, pipes, and protective sheathing for electric cables. The deposition of large black granules, apparently in vacuoles, which corresponded with the reduction of tellurite to amorphous elemental tellurium, has been demonstrated (Gharieb et al., 1999).

The cultivation of fungi in axenic conditions on media enriched with heavy metals such as Pb or Cu leads to an increase of the number and size of metachromatic granules observed with Nomarski DIC optics, as noted in the case of *Paxillus involutus* and *Suillus luteus* (Turnau, unpublished data). The first report on Zn localization within the mycelium of freeze-substituted mycorrhizas demonstrated the presence of this element only in the hyphal cell wall and extrahyphal, polysaccharide slime (Denny and Wilkins, 1987). On the contrary, Bücking and Heyser (1999) have shown that the distribution of Zn depends on the strain of the fungus. Mycelia of *Suillus bovinus* cultivated on media with high levels of Zn accumulated more Zn within the vacuoles, or a similar level of Zn was detected in the vacuolar polyphosphates and within the cytoplasm. In addition, other techniques proved that vacuoles are important in the sequestration of potentially toxic metals; most of these data concern yeasts. Seventy percent of Sr^{2+}, 90% of Mn^{2+}, and 60% of Zn^{2+} accumulated intracellularly by *Saccharomyces cerevisiae* were compartmented in the vacuoles (Nieuwenhuis et al., 1981; Lichko et al., 1982; White and Gadd, 1987; Blackwell et al., 1995). Studies on the kinetics of Cd uptake by *Paxillus involutus*, a mycorrhizal fungus, clearly demonstrated the accumulation of Cd within the vacuoles (Blaudez et al., 2000). In previous studies on this species originating from Cd-rich substratum, carried out on chemically fixed material, this element was most prominently detected within the vacuoles (Turnau et al., 1993b, 1993c).

Microanalytical tools often indicate the co-occurrence of P and heavy metals within the vacuoles. This does not necessarily mean that metals are bound to polyphosphate; they

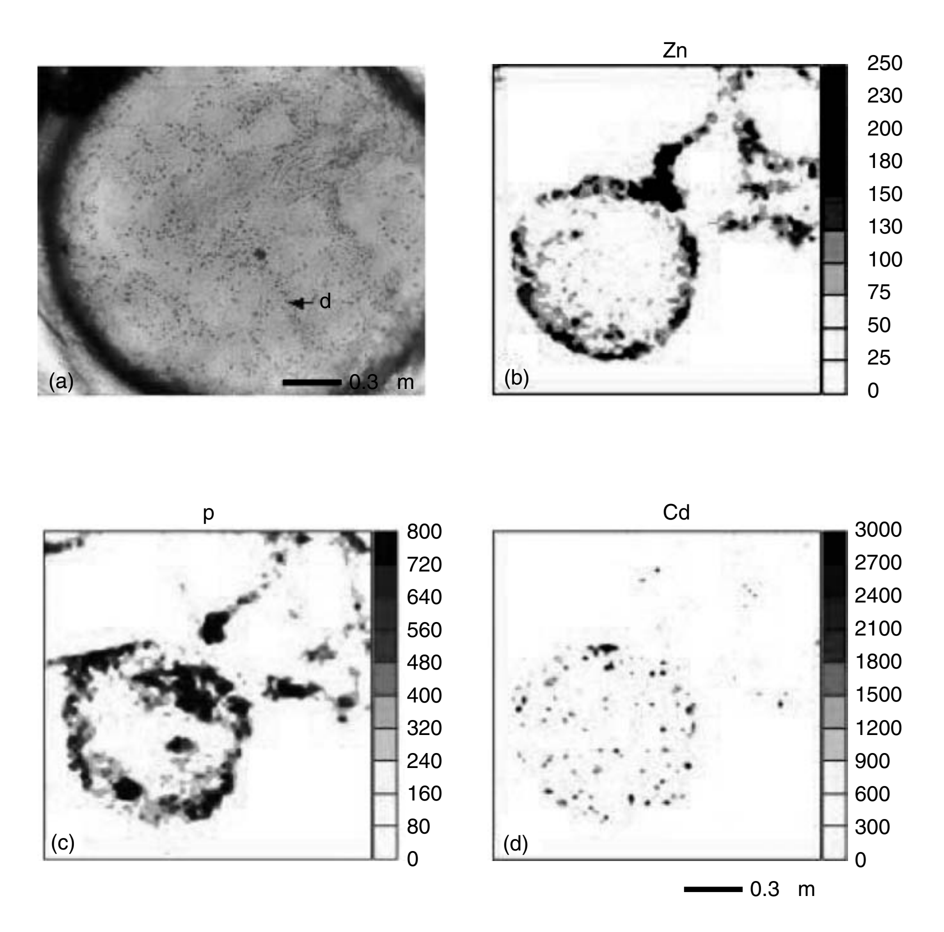


Figure 14.2 **(See color insert following p. 460.)** Metal localization in spores of *Glomus* sp. (a) Spore stained with sodium rhodizonate, suggesting the deposition (d) of heavy metals on the inner surface of the cell wall. (From Turnau, *Acta Soc. Bot. Pol.*, 67, 105–113, 1998. With permission.) (b–d) PIXE elemental maps of spore isolated from polluted soil; concentrations given in mg kg^{-1} (Turnau, Mesjasz-Przybylowicz, and Przybylowicz, unpublished material).

may be bound to OH or SH groups as well. This subject can be further elaborated by EXAFS and ELNES.

14.7 METAL-ACCUMULATING PROPERTIES OF LICHEN-FORMING FUNGI

Epiphytic lichen thalli are commonly used for biomonitoring of air pollution, generating data useful in interpretation of epidemiological patterns of respiratory diseases, due to the fact that they accumulate high concentrations of metals originating almost entirely from the air. Most data on metal content in thalli have been obtained using conventional spectroscopy (see recent reviews: Bennett, 2000; Garty, 2001) or particle-induced x-ray emission (Hrynkiewicz et al., 1979; Olech et al., 1998). In the first case, the metal content is evaluated after acid digestion of samples, while in the second case, the thalli are dried and ground, and a known amount is pressed into a pellet. The lichens are collected from

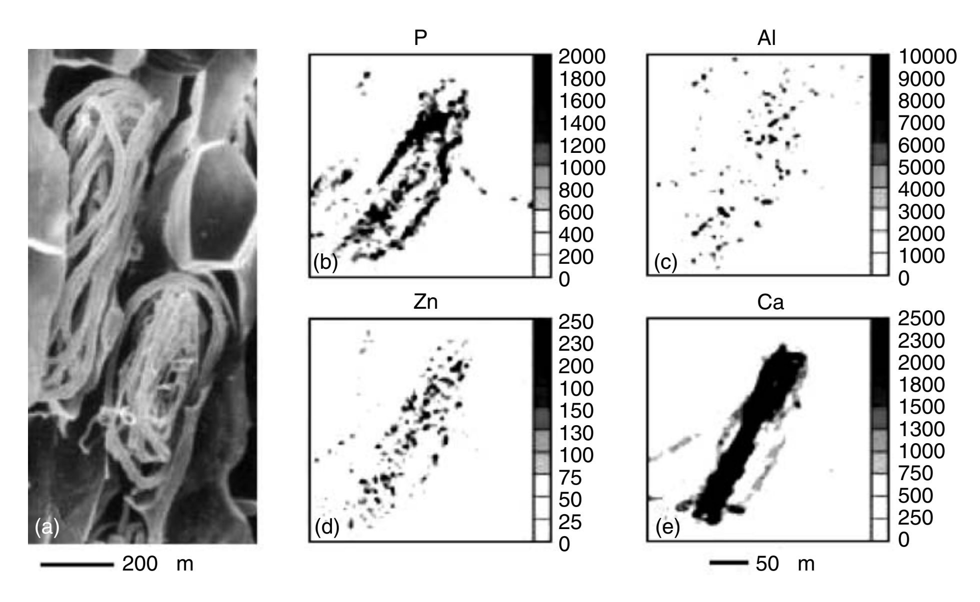


Figure 14.3 **(See color insert following p. 460.)** Element distribution in orchid mycorrhizas. (a) SEM micrograph of fungal coils. (b–e) PIXE elemental maps of coils separated from the plant material; concentrations given in mg kg^{-1} (Turnau, Mesjasz-Przybylowicz, and Przybylowicz, unpublished maps; for more information, see Jurkiewicz et al., 2001).

the site of interest and directly analyzed, or the thalli are collected from relatively non-polluted areas and transplanted for a certain period to the area of interest. Large, age-related differences between peripheral and inner zones of the thalli are often visible. Furthermore, layers of thalli observed in transverse sections differ in compaction, level of gelatinization of the cementing material, thickness of the fungal cell walls, and distribution of various crystals, crystalloids, and hydrophilic wall material (Chiarenzelli et al., 1997). The impact of these differences on element accumulation still remains unresolved. The first attempts were done using the sequential elution procedure to distinguish between extracellular and intracellular uptake (as reviewed by Garty, 2001). These findings were followed by studies with SEM accompanied by EDS (e.g., Garty et al., 1979), x-ray emission spectrography (SEMEX) (Johnsen, 1981), and x-ray fluorescence (XRF) (Olmez et al., 1985). A combination of methods such as x-ray mapping, field emission scanning electron microscopy, light microscopy, chemical spot test, thin-layer chromatography, and Fourier transform infrared spectroscopy allowed the identification of specific accumulation of Pb derived from smelter particles within the fungal tissues of *Acarospora smagardula*, while no Pb was found in the algal zone (Purvis et al., 2000).

Recently, PIXE spectrometry utilizing a focused proton beam has been used to obtain the distribution of elements within thalli of *Xanthoparmelia chlorochroa* (Clark et al., 2001) and *Hypogymnia physodes* (Budka et al., 2002). In both cases, quantitative, two-dimensional element distribution maps for a wide range of elements, including Fe, Ni, Cu, Zn, and As, have been generated. The distribution of oxalates accompanied by increased levels of heavy metals was shown, and the evidence for the transfer of inorganic nutrients across the thalli was presented. In addition, Clark et al. (2001) used SEM and thermogravimetric analyses to characterize the calcium oxalate region, and provided further evidence for the functional role of the oxalate layer, localized between the medulla and the algal layer, in regulation of the water and light regimes.

14.8 HEAVY METAL/MYCORRHIZA INTERACTIONS

The role of mycorrhiza in the uptake and translocation of nutrients has been the subject of a broad range of investigations demonstrating the improved nutritional status of mycorrhizal plants (Harley and Smith, 1983; Jeffries et al., 2003). In several cases, the attenuation of the effect of heavy metals on plants has also been shown (for review, see Gadd, 1993; Hartley et al., 1997; Leyval et al., 1997; Jentschke and Godbold, 2000; Meharg and Cairney, 2000). Developing restoration techniques requires extensive knowledge of the function of mycorrhiza. Relatively much interest has been paid to metal sorption by extraradical mycelia, restricting metal translocation to the host tissues (Jentschke and Godbold, 2000; Joner et al., 2000). Although metal sorption by mycorrhizal mycelia in artificial substrates has been well documented, the significance of this phenomenon under field conditions has not yet been investigated.

Most of the data concerning metal distribution originate from ectomycorrhizal fungi. The individual ectomycorrhizas interact with the soil solution that might contain potentially toxic compounds, and the ability of the fungal mantle to act as a barrier for their penetration is important for root protection. The estimation of the metal content by conventional methods suggests differences in the efficiency of ligand production and heavy metal immobilization among fungal species (Berthelsen et al., 1995; Kottke et al., 1998). Studies involving microanalysis carried out on freeze-dried (Turnau et al., 2001b, 2002) and chemically fixed (Turnau et al., 1996) material have confirmed the diversity among different types of mycorrhizas and demonstrated not only the presence of heavy metals within polysaccharides and pigments of the surface layer of fungal mantle, but also the occurrence of the filtering effect in mycorrhizas such as *Rhizopogon roseolus* and *Suillus luteus* associated with *Pinus sylvestris*. Maps of elemental distribution of freeze-dried mycorrhizas of *S. luteus*, obtained with micro-PIXE, presented quantitative data of metal accumulation. Mycorrhizas of both species are hydrophobic. It is therefore interesting to find out how the metals are able to enter the fungal sheath. Research carried out on *Pisolithus tinctorius*/*Eucalyptus pilularis* mycorrhizas, in which a fluorochrome, 8-hydroxypyrene-1,3,6-trisulphonate (PTS), and lanthanum were traced by x-ray microanalysis, showed that despite the presence of hydrophobins, both the fluorochrome and lanthanum were able to penetrate the mycorrhizas (Vesk et al., 2000). The filtering capacity of *R. roseolus* and *S. luteus* makes them potentially useful for the restoration of industrial wastes. However, further studies are needed to show whether they can indeed be used under field conditions. Both mycorrhizas usually do not make up the most common morphotypes. Although they form very abundant fruit bodies, they are often outcompeted by fungi, which are less effective in metal filtering (Turnau et al., 2002).

Heavy metal distribution has also been studied within endomycorrhizas. The first attempt was done on chemically fixed *Pteridium aquilinum* roots (Turnau et al., 1993b). Heavy metals were detected within the arbuscules. This was followed by observations of freeze-dried arbuscular mycorrhizas of *Plantago lanceolata* (Orowska et al., 2002) and freeze-substituted roots of *Zea mays* (Kaldorf et al., 1999). In the last case, cryosections were studied by laser microprobe and EDS analysis. Secondary ion mass spectrometry has been used to generate data on the distribution of elements within mycorrhizal and nonmycorrhizal roots of maize. The *Glomus* strain used for inoculation was isolated from *Viola calaminaria* and has been shown to alleviate metal toxicity to the host (Hildebrandt et al., 1999). The maps clearly showed the accumulation of Ni, Zn, and Fe within cortical cells in which the arbuscules are usually formed. Furthermore, fewer heavy metals were found within the stele than in the cortex, again suggesting a filtering effect. Similar results have been obtained from roots of *P. lanceolata* cultivated on industrial wastes examined

by PIXE (Orowska et al., 2002). In addition to element distribution maps, reliable data on the concentration of metals within the sample have been obtained. The effectiveness of individual strains to stimulate host growth and metal uptake can be assessed by conventional AAS analysis. However, in field material, several fungal species form mycorrhizal structures within the same roots, and as evidenced by a simple staining with rhodizoniate, they differ in metal-binding effectiveness (Turnau, 1998; Turnau et al., 2001c). The data obtained with PIXE on freeze-dried material also show that heavy metals such as Pb, Cu, and Zn are nonuniformly distributed within the extraradical mycelium, but they show enrichment in places of increased concentrations of K, suggesting the vacuolar compartment (Turnau, unpublished data).

PIXE has also been used to study element distribution in orchid roots that grew abundantly on zinc wastes (Jurkiewicz et al., 2001). Elemental maps showed that Zn and Pb accumulated mostly in the plant root epidermis and fungal coils (Figure 14.3), within the root cortical cells. A statistically significant decrease in Pb and Zn content toward the inside of the root was demonstrated. However, studies carried out with PIXE or SIMS show the transfer of heavy metals into the cortical cells where coils are developed. TEM studies performed on material collected from polluted soils often show the presence of electron dense depositions within plant cells where fungal structures are present. It is possible that these plants degenerate faster due to heavy metal transfer by the fungi.

14.9 CONCLUSIONS

Undoubtedly, the microanalytical techniques contribute to a better understanding of a variety of phenomena in the field of fungal activity. Interdisciplinary cooperation is key to clarifying the mechanisms involved. The assistance of physics is required to correctly handle the techniques. The present state of knowledge already shows a good correlation among results obtained by chemistry, biochemistry, physiology, molecular biology, and microanalysis.

Fungi are unique biological objects because of their intimate interaction with the nonuniform substratum. For this reason, material has to be carefully selected and characterized from the point of view of its physiological and developmental state. There is a wide range of substances that take part in compartmentalization within the fungal mycelium. The strength of binding strongly influences the mobility of elements in the fungal compartments. Different preparation techniques can enable differentiation between mobile and nonmobile elements, and future improvements of the analytical techniques will provide exact characterization of the binding compounds. Microanalytical techniques are powerful and promising tools for further characterization of microscale activity of fungi.

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15

Exploring Fungal Activity with Confocal and Multiphoton Microscopy

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15.1 INTRODUCTION

The opportunity for exploring basic fungal activity from the subcellular to the tissue level has never been greater. A multitude of technological advances in molecular techniques, fluorescent probe chemistry, and optical, computer, and laser technologies have contributed to an exponential growth in imaging for solving critical biological problems. Since its inception, confocal microscopy has proven itself to be an invaluable approach and a whole new way of visualizing biological cells and tissues. The ability to extract high-contrast, high-resolution noninvasive optical sections in both time and space has led to a transformation in how imaging is done in a variety of biological disciplines. Nearly 50 years after its invention, the technology continues to make rapid advancements that expand its utility beyond simple image acquisition. Hardware improvements have been made in all aspects of systems design, including acousto-optical tunable filters; solid-state, diode, and pulsed lasers; use of fiber optics; significant improvements in detection efficiency and the collection of spectral information, to name a few. Multiphoton microscopy has optical sectioning capabilities, much like confocal microscopy, and although these technologies share many attributes and capabilities, multiphoton extends the potential for viewing deeper into samples, is inherently less affected by highly scattering samples, and by virtue of near-infrared (NIR) light source, can be less harmful to living organisms.

This chapter will focus on describing the theoretical and practical implications of confocal and multiphoton microscopy as fundamental tools for solving a host of critical problems in mycological research. In addition, relevant technologies that can take advantage of these powerful research tools will be discussed.

15.2 CONFOCAL MICROSCOPY

In 1955, Marvin Minsky invented the confocal microscope, which was called a double-focusing stage scanning microscope. As a student at Harvard, Minsky used a zirconium arc light source, two objectives with conjugal focal points, a scanning stage, and a surplus military radar scope to generate the first scanning confocal image (Minsky, 1988). In 1960, the first laser was patented and built. However, nearly a decade had passed before the first published report of a laser scanning microscope that incorporated a 5-mW HeNe laser with an x-y-z scanning objective lens (Davidovits and Egger, 1969). Pioneering work continued in several labs (Brakenhoff et al., 1979; Wilson and Sheppard, 1984) with the arrival of the first commercial instruments in the mid-1980s. Ultimately, a series of improved laser and computer capabilities, as well as many other technical advances, led to development of the highly versatile and powerful confocal systems of today.

Confocal microscopy has been described as one of the most significant additions to the field of microscopy in the last century (Blancaflor and Gilroy, 2000). Clearly, its impact has permeated virtually every aspect of biological imaging. Although there are variations in how a confocal image is generated, by far the most common approach is to use laser light focused to a point in a sample (Figure 15.1a). Because a significant number of detailed published reports on the principles of confocal microscopy are readily available, an in-depth review of the inside workings of a confocal microscope will not be provided here. For more comprehensive treatments on the subject, see the following recommended reading: a compilation of patents and publications related to confocal microscopy (Masters, 1996), an excellent evaluation of a variety of critical parameters for optimizing confocal performance (Zucker and Price, 2001), and the *Handbook of Biological Confocal Microscopy* (Pawley, 1995). Reviews on confocal microscopy specifically addressing applications in mycological research, including basic theoretical considerations of lateral and axial resolution, optical section thickness, and z-sectioning, are also available (Kwon et al., 1993; Czymmek et al., 1994).

15.3 MULTIPHOTON MICROSCOPY

Multiphoton fluorescence microscopy is a powerful technology that enables the acquisition of optical sections without the use of the pinhole aperture typically used for confocal microscopy. Because this technology is relatively new, some emphasis will be placed on aspects of image formation and how multiphoton microscopy compares with confocal microscopy. The two-photon principle was first described by Goeppert-Mayer (1931). The potential of this concept for imaging was initially reported by Sheppard and Kompfner (1978) and finally realized 12 years later in cultured cells stained with Hoechst 33258 (Denk et al., 1990). The two-photon effect occurs when a fluorescent molecule simultaneously absorbs two photons, producing an electronic transition from the ground to excited state equal to two times the energy of each incident photon (Figure 15.2). For example, the simultaneous absorption of two red photons (each 980 nm) can yield an electronic transition equivalent to a single blue photon (490 nm).

Although the excitation properties of a fluorescent molecule are different for single-photon and multiphoton events, the emission spectrum remains unchanged, regardless of how the electronic transition occurred (Xu and Webb, 1996; Xu et al., 1996). Under specific imaging conditions, a multiphoton effect can be induced with more than two photons (e.g., three-photon excitation; Maiti et al., 1997). For the sake of brevity, this discussion will

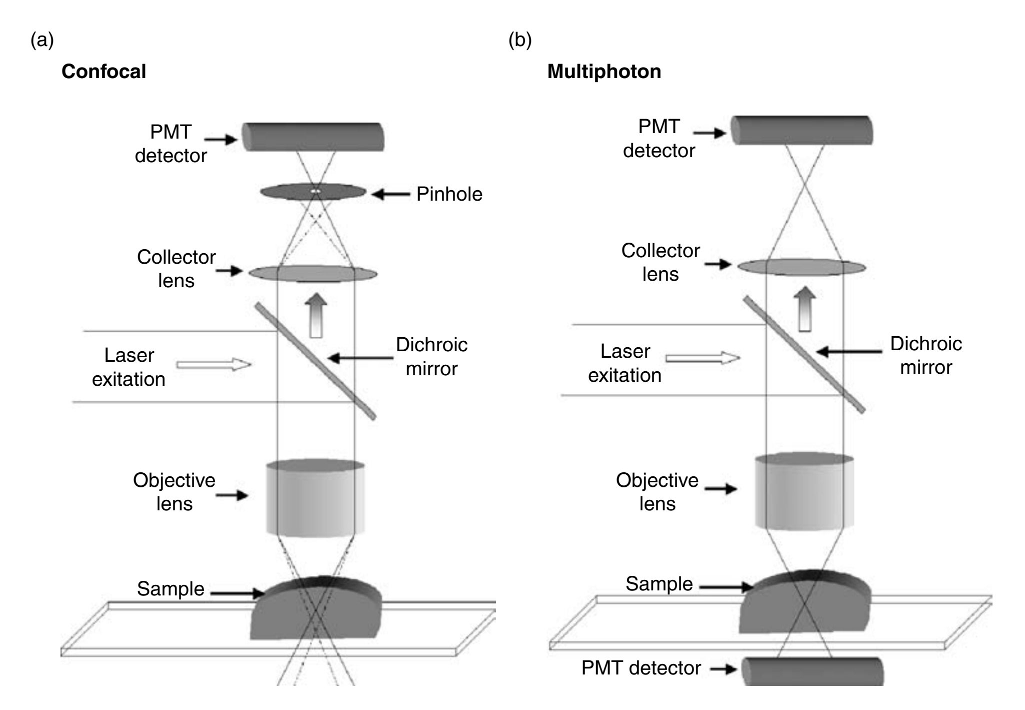


Figure 15.1 Simplified light path of laser scanning confocal (a) and multiphoton (b) microscopes. (a) Laser light focused via an objective lens to a diffraction-limited spot in a labeled sample will excite fluorescence. Any fluorescence generated from the spot returning through the objective lens is then focused via a collector lens to a conjugal focal point at a pinhole (gray-filled solid lines). The out-of-focus fluorescence signal generated by the cone of light above or below the focal plane (dashed lines), as well as scattered light, will not come into focus at the pinhole and will be blocked from the detector. Because the laser is scanned in a raster pattern across the sample (using linear mirror galvanometers), a digital image is created with each pixel representing a single coordinate during the scan. The resulting image is an optical section. (b) Multiphoton imaging is achieved using mode-locked near-infrared lasers that produce short-duration pulses (~100 femtoseconds) and high peak powers. The rapidly pulsed near-infrared light is focused to a diffraction-limited spot by a high-numerical-aperture objective lens. Because the multiphoton effect has a quadratic dependency on illumination intensity, the likelihood of such an event occurring outside the objective focal spot decreases rapidly along the beam path. Hence, an inherent confocal image is generated by raster scanning the spot across the specimen of interest. Because the fluorescence signal emanates from only a small volume (defined by the NA of the objective lens), no pinhole is required and detectors can be positioned in a variety of locations, including the transmitted light path.

be limited to two-photon excitation, and two- or three-photon excitation will be referred to collectively as multiphoton. For a two-photon event to occur, a significant density of incident photons is required. This typically is achieved using ultrafast, mode-locked near-infrared lasers (e.g., Ti:sapphire, 100- to 200-femtosecond pulse duration, 76-MHz repetition rate, one pulse every 13.2 nsec). Under the appropriate conditions, these lasers produce short-duration pulses with the high peak power required for a multiphoton effect and an average power low enough to make specimen damage negligible. Such lasers are typically tunable over a range of ~700 to 1000 nm, which permits optimal wavelength selection to elicit an efficient multiphoton effect. The rapidly pulsed near-infrared light is focused to a diffraction-limited spot by a high-numerical-aperture (NA) objective lens.

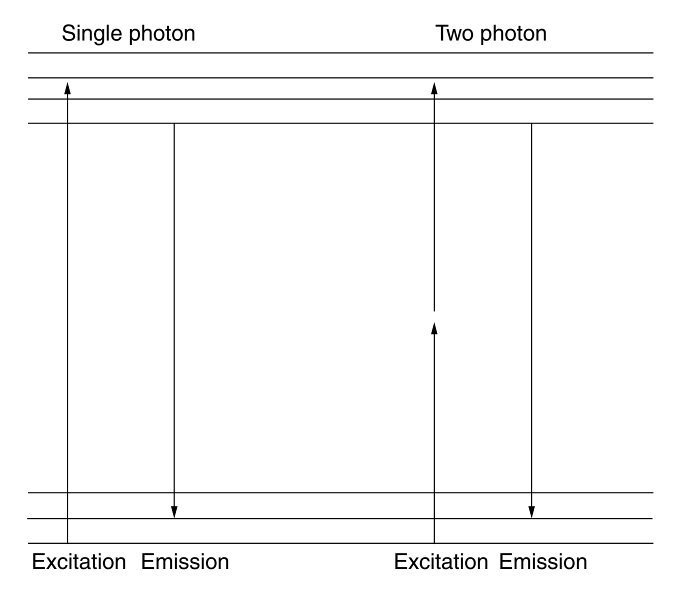
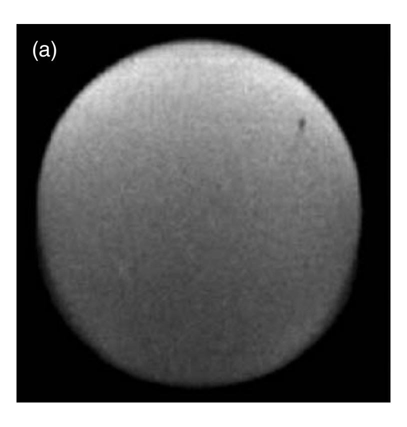


Figure 15.2 Jablonksi diagram illustrating the absorption and emission of light, comparing an electronic transition with single-photon and two-photon events. When a fluorophore absorbs the appropriate wavelength of light, it is excited from the ground state to a higher-energy level. With fluorescence, when the molecule returns to the ground state, a photon having lower energy than the incident photon is emitted. In this diagram, a two-photon electronic transition required the instantaneous absorption of two lower-energy photons to achieve the same electronic transition as the higher-energy single-photon event.

The actual volume of this spot can be as small as 0.1 femtoliters (μm^3) for a high-NA (1.4) lens (Denk et al., 1995). Because the multiphoton effect has a quadratic dependency on illumination intensity, the likelihood of such an event occurring outside the objective focal spot decreases rapidly along the beam path (z-axis). Hence, an inherently confocal image is generated by raster scanning the laser spot across the specimen.

Hybrid confocal/multiphoton systems share many optical components (compare Figure 15.1a and 15.1b). Fluorescence generated using multiphoton excitation may be collected following the same light path as the confocal signal, but the pinhole typically would be opened to its widest position, maximizing signal collection. This is referred to as descanned detection because the emission signal passes back through the same scanning mirrors used for laser excitation. Alternatively, external detectors may be placed in appropriate positions to collect more light in a nondescanned configuration. In Figure 15.1b, a detector is placed below the sample in the transmitted light path. Such a configuration would potentially improve overall sensitivity by minimizing the number of optical elements in the collection pathway. However, this approach is also more likely to have problems with stray light from room lights, monitors, etc., and the microscope must be shielded as much as possible. In order to circumvent this, a relatively simple system modification for nondescanned detectors, based on the principle of phase-sensitive demodulation of the pulsed laser, permits clean separation of multiphoton images in the presence of ambient room light (Fisher et al., 2002).

In specific situations, multiphoton microscopy has several advantages over conventional fluorescence and confocal imaging:



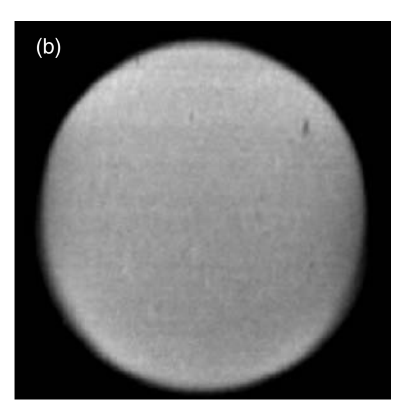
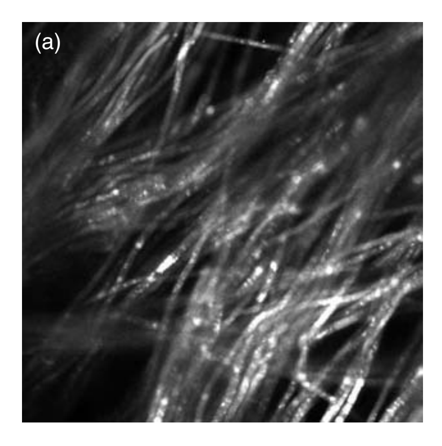


Figure 15.3 Comparison of confocal (a) and multiphoton (b) imaging within a thick specimen. XZ images of the same FITC-lysozyme-loaded 100-μm-diameter sepharose bead using confocal and multiphoton microscopy illustrated the improved signal collection deep within the sample using multiphoton microscopy. The top of the image was the direction from which the incident laser originated. (Images provided courtesy of S. Dziennik and A. Lenhoff.)

1. Under the appropriate power (usually 3 to 5 mW at the specimen), near-infrared light is far less damaging to many living samples than visible and UV light (Stelzer et al., 1994).
2. Lower-energy near-infrared light is scattered less; thus, imaging deeper into highly scattering thick specimens is possible (Figure 15.3 and Figure 15.4).
3. No confocal pinhole is required; therefore, the emission signal collection is more efficient.
4. Photobleaching is restricted to the focal plane rather than throughout the beam path, as with confocal microscopy (Figure 15.5).



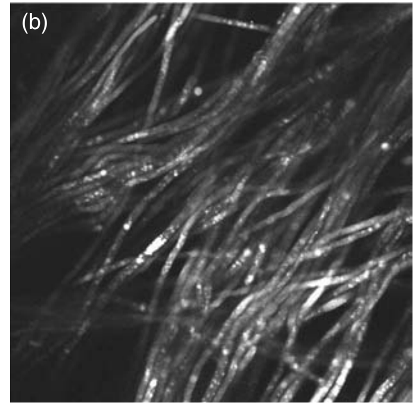
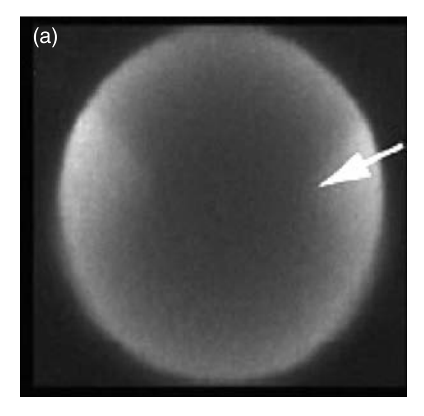


Figure 15.4 Comparison of confocal (a) and multiphoton (b) imaging within a thick fungal specimen. (a) *Fusarium oxysporum* hyphae expressing ZsGreen fluorescent protein can be imaged 100 μm into nutrient agar using confocal microscopy. However, regions of blurriness due to scattered light effects can be seen. This phenomenon is most often observed when overlying hyphae are present. (b) Multiphoton image at the same optical plane as image A demonstrating improved imaging deep within the specimen.



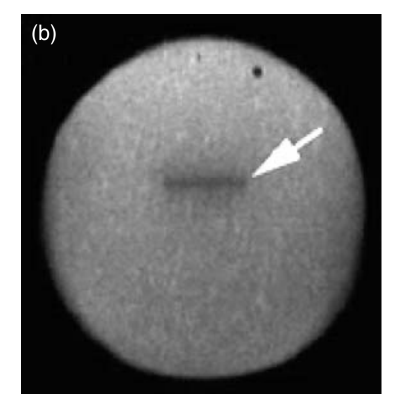


Figure 15.5 Comparison of confocal (a) and multiphoton (b) imaging after photobleaching. XZ images of the same fluorescein isothiocyanate (FITC)-filled 100-μm-diameter sepharose beads photobleached using confocal and multiphoton microscopy. In confocal mode (a), photobleaching a single plane (arrow) within the sepharose bead demonstrated that the fluorophore was considerably bleached above and below the plane of focus. In multiphoton mode (b), photobleaching was clearly restricted to the narrow plane of focus (arrow). (Images provided courtesy of S. Dziennik and A. Lenhoff.)

5. Phototoxic effects are essentially limited to the focal plane.
6. Inner filter effects due to absorption of incident laser light by fluorescent molecules before the plane of focus are eliminated.
7. Multiphoton excitation can provide highly localized spatial control (on the order of femtoliters) for fluorescence recovery after photobleaching (FRAP), photoactivation, and uncaging experiments.

Even with its many advantages, a number of practical considerations must be weighed when choosing multiphoton microscopy over confocal or other conventional imaging methods, namely, whether the imaging requirements include one or more of the advantages described above. If this is the case, a good knowledge of other important factors involved with generating optimal multiphoton image formation is beneficial and will be described further.

One distinct difference between confocal and multiphoton microscopy is reduced resolution. Principally, due to lower-energy near infrared radiation (NIR) light forming a larger diffraction-limited spot at the objective focal plane, the resolution of two-photon vs. one-photon (confocal microscopy) excitation for the same fluorophore results in an approximately twofold decrease in resolution for two-photon excitation (Denk et al., 1995; Wolleschensky et al., 1998). However, when a confocal aperture is used in conjunction with multiphoton excitation, a nearly 50% improvement in multiphoton resolution can be achieved (Stelzer et al., 1994). Although resolution enhancement is gained by incorporating a pinhole, usable signal is rejected and system sensitivity is sacrificed.

Multiphoton systems, like UV confocal microscopes, are expensive primarily due to the need for specialized high-power lasers. They also require specific optical coatings to allow efficient laser transmission at NIR wavelengths throughout the system and a collimation lens to bring the NIR laser coincident with any visible lasers when performing simultaneous multiphoton and confocal imaging. Although some lenses have been designed with exceptional NIR throughput, many standard objective lenses have been optimized for visible or UV transmission and require significantly more laser power to

achieve a multiphoton effect. Fortunately, most direct coupled lasers have sufficient power at the lower and upper ends of the tuning curve, where the lower laser output can be more challenging. Fiber-optic laser delivery, which has shown great utility with continuous wave-visible and UV lasers, poses special difficulties with multiphoton systems. This is in large part due to decreased peak power, a restricted range of tunable wavelengths, and sensitivity to misalignment. Some progress has been made in this area (Helmchen et al., 2002); however, the vast majority of multiphoton systems are direct coupled, facilitating ease of use. With the above-mentioned laser throughput issues, it is advisable to empirically evaluate available lenses for a given system and specimen. Although not directly a throughput concern when tuning at NIR wavelengths, water absorption peaks at 760, 820, and 900 nm, and strongly between 920 and 980 nm, make the attainment of mode lock (pulsed laser light) problematic unless the laser cavity is purged with dry nitrogen gas. In addition, significant absorption is still possible in the aqueous sample environment, resulting in increased and potentially harmful localized heating effects (Konig et al., 1996). Such heating effects may also occur with increased laser excitation powers and with NIR-absorbing molecules or media (e.g., melanin and salts). Tirlapur and Konig (1999) used this heating phenomenon to their advantage in *Arabidopsis* root meristematic cells by focusing the multiphoton NIR laser to the plasma membrane of individual cells, thus increasing subsequent uptake of propidium iodide. Any cell that was coupled to the targeted cell would also incorporate the dye, providing some information about which cells in the meristem were linked cytoplasmically.

A major optical effect that is not a concern with confocal microscopy is related to pulsed laser light. When a light pulse propagates through optical elements (e.g., the objective lens), the higher-frequency components travel more slowly than the lower-frequency components and the pulse becomes "chirped" or frequency swept (Helmchen et al., 2001). This phenomenon is also known as group velocity dispersion, and the result is a temporal spreading of the pulse that can cause a considerable reduction in peak intensity, and hence the multiphoton effect (Fan et al., 1999). The dispersion properties of a given objective lens (and other optical components) can result in significant pulse broadening, which can be compensated for if the system is configured to do so (Guild et al., 1997).

The ability to tune most multiphoton lasers is advantageous, since the optimal excitation wavelength for a given fluorescent molecule can be selected, as opposed to single-photon confocal, where at best several discrete wavelengths are available. However, one common difficulty associated with multiphoton microscopy is determining the optimal excitation wavelength. This problem can be exacerbated by the fact that some fluorescent molecules have poor multiphoton absorption cross sections. The multiphoton absorption cross section (expressed in Goppert-Mayer (GM) units: 1 GM = 10^{-50} cm^4 sec/photon) (Kiskin et al., 2002) indicates the absorption maximum and how well a fluorophore is excited at a given wavelength. Ideally, the simple doubling of the one-photon excitation maximum would yield the appropriate two-photon maximum, and this is a good approximation for a number of fluorescent probes, such as rhodamine B, fluorescein, DiI, and lucifer yellow (Xu and Webb, 1996). However, in many cases, this rule does not apply due to vibronic coupling (Xu et al., 1996). Recent developments in automated tunable lasers will permit the rapid generation of two-photon excitation characteristics of individual fluorescent molecules with reasonable accuracy, eliminating this as a complicating factor when using new or uncharacterized fluorophores or unknown tissue-related fluorescence. In addition, laser operation will be greatly simplified, as manually tuned lasers require specific, in-depth training for proper use.

The fact that multiphoton excitation can interact as a single-photon event in a discrete volume presents many intriguing possibilities for uncaging and photoactivation techniques.

Photoactivatable green fluorescent protein (GFP) (PA-GFP) is basically nonfluorescent when excited with 488-nm laser light, but become 100 times more fluorescent after irradiation with UV light (Patterson and Lippincott-Schwartz, 2002). PA-GFP molecules can be easily and specifically photoactivated in user-defined areas of the cell. Spatial and temporal changes in tagged molecules can be followed after activation. To date, no published reports have demonstrated PA-GFP with multiphoton excitation. However, my lab, as well as others (George Patterson, personal communication), has successfully activated PA-GFP in mammalian cells, and it is likely that published reports will soon follow. Successful uncaging of caged compounds has been an area of sporadic success. Caged compounds that would normally be readily cleaved upon UV irradiation are poorly released with the appropriate multiphoton excitation. Efforts have been made to find improved multiphoton caged compounds (Albota et al., 1998). However, Kiskin et al. (2002) reported that caged compounds would require a 31-GM cross section to be physiologically useful, and that current caged compounds are only a few GM at best; hence, the laser powers required to uncage existing compounds would be harmful to cells. With that said, photoactivation and photolysis of caged compounds using the precision of multiphoton excitation, if and when available, would provide added capabilities that cannot be realized to the same extent with confocal microscopy.

The usefulness of multiphoton microscopy for reducing photobleaching varies. Even though photobleaching is restricted to the objective focal plain with multiphoton imaging (Figure 15.5b), bleaching rates at higher-incident laser powers can greatly exceed those observed with confocal microscopy (Patterson and Piston, 2000). However, the authors point out that this effect is greatly reduced and very manageable when using the lower powers typically used to image live cells. Ultimately, when imaging near the coverslip, where scattered light events are less pronounced, confocal microscopy would likely be the best choice for many fungal samples. However, imaging deeper into highly scattering tissues can result in a rapid drop in the signal-to-noise ratio, with a concomitant increase in spherical aberration for confocal imaging. In such cases, the image degradation noted in confocal microscopy can be alleviated with multiphoton microscopy, markedly improving image quality (Figure 15.4 here and Figure 2 in Howard, 2001).

15.4 SPECTRAL IMAGING

Recently, spectral confocal/multiphoton microscopes have become commercially available. The ability to derive spectral information from samples has significant implications for fungal research. There are several approaches to obtain spectral data from a sample using a grating, prism, or series of long-pass and band-pass filters (Haraguchi et al., 2002). Spectral imaging is not limited to confocal or multiphoton microscopy and can be used in conjunction with conventional fluorescence as well, resulting in defined spectral regions collected by a single detector or array of detectors. A specified total range of collected fluorescence (for example, 500 to 600 nm) could consist of 10 images, each representing 10-nm windows of the spectrum. Intensity values at any given pixel or group of pixels can be plotted over the selected range, providing an emission curve. In the case of multiple probes, defining a fingerprint for each individual fluorophore in a single-label experiment results in a reference spectrum that can be used to cleanly separate even closely overlapping fluorophores by a process called linear unmixing (Hiraoka et al., 2002). Figure 15.6 illustrates an example of raw data collected from a mixture of three strains of *Fusarium*, each transformed to express a different reef coral fluorescent protein. In this case, the

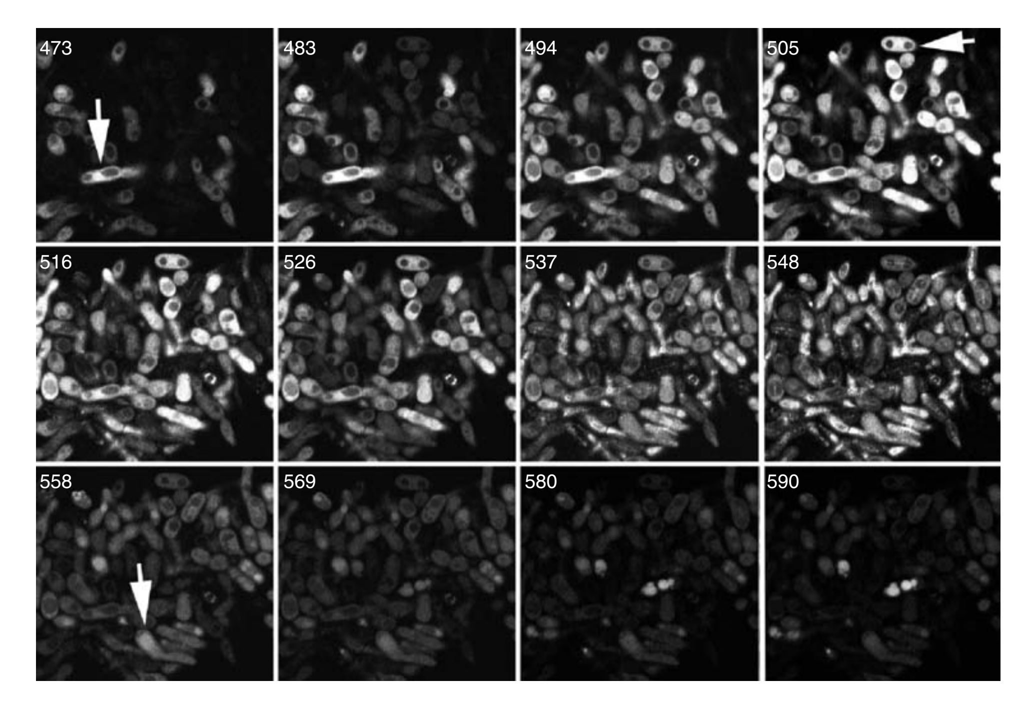


Figure 15.6 Spectral confocal microscopy of fluorescent proteins. Cytoplasmic expression of the fluorescent proteins AmCyan, ZsGreen, and ZsYellow resulted in significant fluorescence emission overlap that was readily separated in germinating conidia of *Fusarium* using spectral confocal and linear unmixing techniques. This data set was acquired on an LSM510 META confocal microscope using 12 channels (10.2-nm lambda width per channel) of a 32-channel photomultiple tube (PMT) array ranging from 473 to 590 nm (channel center points). The arrows depict spores expressing AmCyan (473 nm), ZsGreen (505 nm), and ZsYellow (558 nm). Note the intensity changes for these spores as you compare adjacent images in the lambda series. See Figure 15.7 for umixed results.

emissions from the three fluorescent proteins overlap significantly, making it problematic, if not impossible, to cleanly separate using conventional filters (Bourett et al., 2002), but were easily separated following linear unmixing (Figure 15.7a). Although it is preferred to select fluorophores that avoid such spectral overlap, this is not always possible. For example, the fluorescent probes Calcofluor for chitin and 4′,6-diamidino-2-phenylindole (DAPI) for nuclei (or GFP and YFP) are extremely useful fungal cellular markers that have overlapping excitation and emission characteristics that could be cleanly separated by this approach. Another notable advantage of spectral imaging is that a reference spectrum from tissue autofluorescence (e.g., wood and other plant tissues or growth media) can be acquired and separated from the desired fluorescent probe signal. The autofluorescent signal need not be discarded because it can provide details about the surrounding sample. Autofluorescence is often broad spectrum, making it very difficult to eliminate by standard filters. A spectral scan of several species of cultured hyphae (Baschien et al., 2001) proved invaluable for selecting probes whose emission wavelengths avoided insidious autofluorescence with *in situ* hybridization experiments. Spectral imaging has also been shown to be advantageous when using techniques such as fluorescence resonance energy transfer (FRET) for imaging calcium in mammalian cells with cameleon-2 (Hiraoka et al., 2002). Typically, FRET pairs are spectrally close and suffer from bleed-

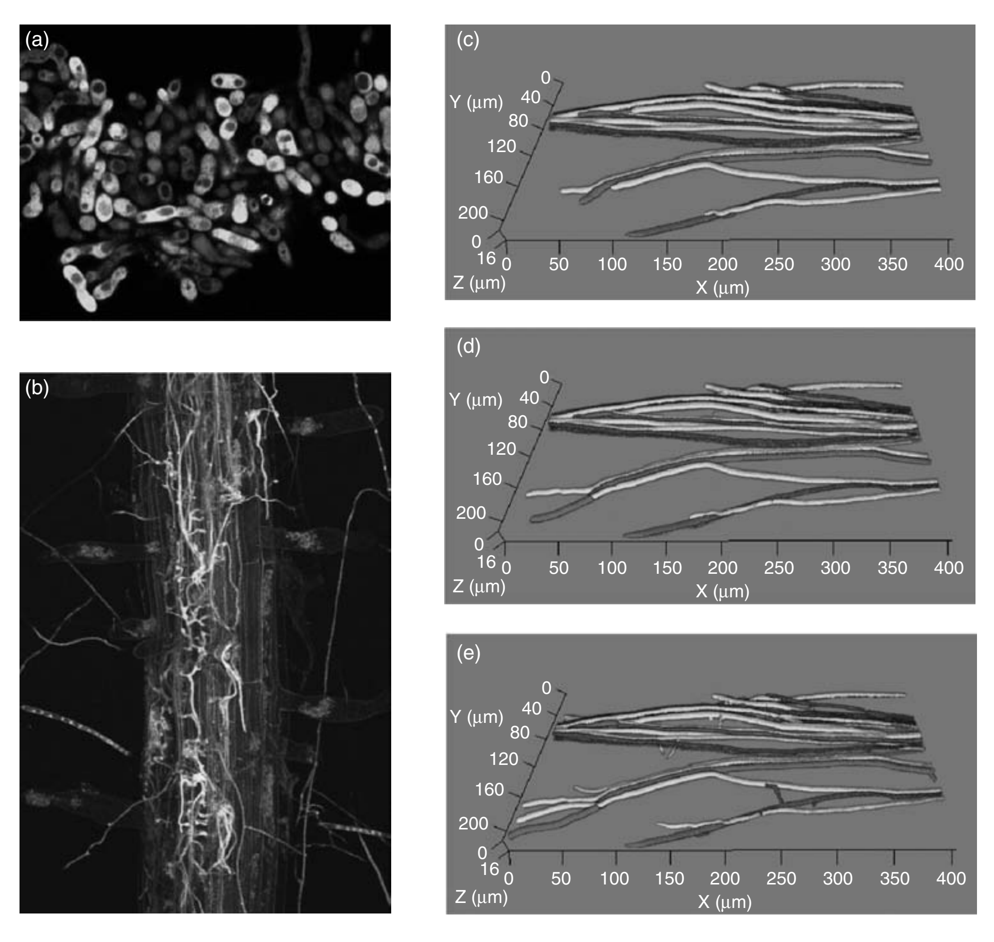


Figure 15.7 **(See color insert following p. 460.)** (a) Spectral confocal techniques allowed clear separation of closely overlapping fluorescent molecules such as AmCyan (blue), ZsGreen (green), and ZsYellow (red) following linear unmixing. (Images provided courtesy of K. Czymmek, T. Bourett, J. Sweigard, and R. Howard.) (b) *In vivo* constitutive cytoplasmic expression of the reef coral fluorescent protein ZsGreen in *Fusarium oxysporum* was used to monitor disease progression in *Arabidopsis* during root infection. (Images provided courtesy of K. Czymmek, J. Sweigard, M. Fogg, and S. Kang.) (c–e) Four-dimensional (three-dimensions over time) series of cytoplasmic-expressing ZsGreen and AsRed *Fusarium* hyphae as they interact in culture. These three-dimensional stacks were selected from a four-dimensional data set (T = 0, 32, and 72 min) that monitored hyphal growth every 8 min over a 3-h period. (Images provided courtesy of V. Cooke and K. Czymmek.)

through problems, necessitating specific postacquisition corrections. By virtue of linear unmixing spectral sequences, clean separation of donor and acceptor fluorescence can be faithfully obtained, minimizing these bleed-through artifacts. To date, there are only a few examples of spectral confocal/multiphoton microscopy applied to mycological problems (Baschien et al., 2001; Bourett et al., 2002) — a reflection of the very recent introduction of this technology. There is little doubt that multispectral imaging will become invaluable as a tool for clean separation of probed fungal structures and byproducts from their complex environment.

15.5 SUPPORTING TECHNOLOGIES

The state-of-the-art confocal/multiphoton microscopy is also tied to concomitant advances in fluorescence-based technologies. One such example, Alexa Fluor dyes, is significantly brighter and far more photostable than previous generations of fluorophores (Panchuk-Voloshina et al., 1999). This is typically an advantage; however, it can sometimes be problematic for photobleaching or FRET studies because the dyes can be very difficult to photobleach. Another technology, called Zenon, allows several primary antibodies against the same species (e.g., mouse) to be easily applied in a single step for multilabel experiments in a direct labeling approach (Molecular Probes, Eugene, OR). Quantum dots are semiconductor nanocrystals that pose interesting possibilities for a host of applications. These small particles, on the order of a few nanometers in size, have very discrete emission wavelengths depending on subtle differences in size (Seydel, 2003). This feature alone would lead to very exciting potential for multilabeled experiments, as one could conceive of labeling six or more targets in the same cell simultaneously. But this is not the only useful feature of quantum dots; they are also extremely resistant to photobleaching and exceptionally bright (Larson et al., 2003), making them well suited to experiments where sensitivity is an issue (e.g., *in situ* hybridization localization of low-abundance molecules). In addition, it has been reported that quantum dots have a two-photon cross section of 47,000 GM, which is two to three orders of magnitude greater than any existing fluorescent probe (Larson et al., 2003). This is incredibly bright and means that individual dots could likely be seen at very low laser energy input and conceivably imaged with low-power objective lenses. Although only a few spectral versions of quantum dots are commercially available (specially treated to remain stable in the hydrophilic environment typically found in biological systems), it is expected that a growing repertoire will be offered in the near future. It is less clear how these could be used in living cells, but they may have potential for studying endocytosis.

Other advanced fluorescence-based imaging techniques are readily adaptable to laser scanning confocal microscopy, such as FRET (Bastiaens and Jovin, 1997; Gadella et al., 1999), fluorescence lifetime imaging microscopy (FLIM) (Pepperkok et al., 1999), fluorescence correlation spectroscopy (FCS) (Schwille et al., 1999), fluorescence recovery after photobleaching (FRAP) (Siggia et al., 2000; Brandizzi et al., 2002), fluorescence loss in photobleaching (FLIP) (Judd et al., 2003), fluorescence localization after photobleaching (FLAP) (Dunn et al., 2002), photoactivatable GFP (Patterson and Lippincott-Schwartz, 2002), and kindling fluorescent protein (KFP) (Chudakov et al., 2003). Many of these techniques (e.g., FRAP, FLIP, FLAP, KFP) can be used with modern confocal/multiphoton systems without additional or expensive hardware upgrades.

15.6 MYCOLOGICAL APPLICATIONS

A rapidly growing body of literature clearly illustrates the power of optical sectioning approaches for investigating fungi *in vitro*, as well as with interactions in exceptionally multifaceted and diverse environmental situations. The relative ease in which discrete, high-resolution image planes can be acquired, increased accessibility to confocal microscopes in many institutions, and the amenability of fungi to optical microscopy in general have played important roles in their popularity and success. From single optical sections of fixed material to multidimensional, multichannel, or multispectral imaging of living hyphae, confocal and multiphoton microscopies have proven to be invaluable as research tools. The remainder of this chapter, although not exhaustive and primarily focused on filamentous

fungi, will explore the major areas in which investigators have already used the sophisticated capabilities of multiphoton/confocal microscopy in a multitude of applications.

15.6.1 Immunofluorescence

Some of the earliest reports of confocal microscopy for fungal applications used immunofluorescence techniques (Hardham et al., 1991; Kwon et al., 1991) and have been previously reviewed (Kwon et al., 1993; Czymmek et al., 1994). These reports serve as useful starting points if considering fluorescence-based antibody localization in fungi. Due to the availability and relative ease in which suitable antibodies can be created and applied for immunofluorescence microscopy, this methodology has become relatively commonplace in the literature. For example, antibodies were raised against a series of cellulolytic enzymes, and their distribution in cultured *Volariella* hyphae was documented via confocal microscopy (Cai et al., 1999). Wood autofluorescence, which posed a significant challenge when using conventional microscopy, due to the obscured visibility of labeled structures, easily delineated antibody-labeled *Ophiostoma* hyphae in thick-sectioned pine wood samples (Xiao et al., 1999). Another interesting approach was the correlation of the opportunistic pathogenic yeast *Candida albicans* (loaded with FITC) and actin in human cell lines (Tsarfaty et al., 2000). In this case, the authors used colocalization analysis and optical sectioning in the z-axis to illustrate that actin specifically assembled near *Candida* interaction sites.

Antibodies raised against any number of antigens (in conjunction with a fluorescent tag) can be used to localize specific cellular proteins of interest. However, due to their size, unless the antigen is on the cell surface or otherwise introduced (e.g., microinjection), employing antibodies for immunofluorescence typically requires fixed and permeabilized cells. A number of protocols exist for antibody labeling of fungal samples. The method of choice is influenced by a variety of factors, including the particular antigen, subcellular compartment, and variations in cell wall biochemistry. A simple approach using freeze-substitution fixation and methacrylate de-embedment, thus avoiding sectioning and wall digesting enzymes in filamentous fungi, has been described (Bourett et al., 1998). A major advantage with this technique is that whole-mount fungi can be imaged in three dimensions using confocal microscopy with high-fidelity cryogenic preservation. Others have used a slightly modified version of this method to monitor microtubule and nuclear behavior in ropy-1 mutants of *Neurospora crassa* with similar success (Riquelme et al., 2002), or just freeze-substitution and methanol fixation to visualize the tubulin and actin cytoskeleton in the chytridiomycete *Allomyces* (McDaniel and Roberson, 2000). Regardless of the permeabilization tactic used, immunofluorescence is now routine and a core competency of confocal/multiphoton microscopy.

15.6.2 *In Situ* Hybridization

Although examples of immunolabeled proteins are abundant in the fungal literature, very little data are available using *in situ* hybridization for nucleic acid detection. Li et al. (1997) quantified fluorescence of *Aureobasidium pullulans* on slides and leaf surfaces counterstained with propidium iodide; Baschien et al. (2001) were successful with hybridization of freshwater fungi; and Sterflinger and Hain (1999) demonstrated hybridization in black yeast and meristematic fungi. Major causes for the scarcity of hybridization data include lack of sensitivity, difficulty with reliable fungal cell wall permeabilization, and RNA degradation by wall digesting enzyme cocktails, as alluded to previously (Bourett et al., 1998). When optimized and broadly applicable approaches become available for fungi (e.g., using peptide nucleic acid probes or polymerase chain reaction (PCR)), hybridization methods are likely to derive similar benefits with confocal/multiphoton microscopy as have other fluorescence probe techniques.

15.6.3 Affinity and Vital Fluorescent Probes

As mentioned previously, confocal microscopy is amenable to the vast majority of fluorescence-based applications. One area in particular that has yielded significant benefits is live cell imaging. This has in large part been spurred on by the ever-increasing availability of vital fluorescence markers that can quickly and easily label a broad range of fungal structures, compartments, and molecules of interest. In its simplest forms, a classic dye such as acridine orange can be applied as a pH indicator to localize acidic compartments, as with *Fonsecaea pedrosoi* (Franzen et al., 1999), and fluorescent probes can be used to determine cell wall porosity (FITC-Dextran) or membrane integrity (propidium iodide, 5 (and 6)-carboxy-2′,7′-dichlorofluorescein diacetate (CDFDA), and FUN1) (Brul et al., 1997). Others have imaged the inherently autofluorescent arbuscular mycorrhizal (AM) fungus *Gigaspora* in direct association with *Rhizobium* and *Pseudomonas* labeled with live or dead bacterial viability probes (Bianciotto et al., 1996) or Congo Red for the estimation of fungal biomass in a fermentation system (Nopharatana et al., 2003).

A poorly understood area of intense interest is the nature of the dynamic trafficking of vesicles involved with endo- and exocytosis in the Spitzenkorper at the hyphal apex (Read and Hickey, 2001). Application of a series of fluorescent probes (viz., FM4-64, Lucifer yellow carbohydrazide, FITC-Dextran) in germinating conidia of *Magnaporthe grisea* showed that FM4-64 was especially useful for observing endocytotic events (Atkinson et al., 2002). Other FM4-64 work from this lab involved labeling live cells from nine phylogenetically distinct fungi and demonstrated putative endosomal trafficking and localization at the Spitzenkorper and satellites (Read et al., 1998; Fisher-Parton et al., 2000). FM4-64 is quite versatile and also has been used to follow membrane development during zoospore formation in *Allomyces* (Fisher et al., 2000) and for vegetative hyphal fusion in *Neurospora crassa* (Hickey et al., 2002). Whether used to investigate endocytosis (Fisher-Parton et al., 2000), the endoplasmic reticulum (Cole et al., 2000), mitochondria (Bobek and Situ, 2003), actin (Jackson and Hardham, 1998), pH (Parton et al., 1997), or vacuoles (Cole et al., 1998), vital dyes have unmistakably established their utility for probing the fungal cell (Spear et al., 1999). As an interesting extension to vital probe imaging, Hyde et al. (2003) demonstrated the immobilization and retention of the vital dye chloromethyl aminocoumarin (CMAC) with multiphoton following freeze-substitution fixation in tetrahydrofuran and subsequent infiltration with Spurr's resin. They also applied this method with varying success to 5-chloromethylfluorescein diacetate (CMFDA), Oregon Green 488 carboxylic acid diacetate (carboxy-DDFDA), ER-Tracker (endoplasmic reticulum), Bodipy BFA (endoplasmic reticulum), and MY-64 (vacuole membrane)-loaded cells. It was suggested that a similar approach might prove beneficial for analysis of GFP-expressing cells, although fluorescent proteins are typically quenched by solvents unless present in high concentrations.

There is little doubt that Ca^{2+} plays an important role in many aspects of fungal development, growth, and physiology (Hyde, 1998). Hence, a number of investigators have sought to explore calcium distribution and dynamics in living hyphae (Hyde, 1998; Read et al., 1998 and references therein). However, technical issues unrelated to confocal or multiphoton microscopy (Stricker and Whitaker, 1999) have made cytosolic localization problematic. In fact, these issues are not strictly a calcium dye phenomenon but exist for a variety of probes. The size and charge of a fluorophore not only play an important role in whether it can be loaded into a given cell, but also influence where it may be compartmentalized. Such difficulties have been alleviated partially by the creative use of fluorescent proteins. For example, Ca^{2+} has been imaged using cameleon biosensors that employ a Ca^{2+}-sensitive calmodulin-M13 complex that bridges the fluorescent proteins enhanced cyan fluorescent protein (ECFP) and enhanced yellow fluorescent protein (EYFP) (Miyawaki et al., 1997). When free calcium binds to this fusion protein, a conformational

change in the molecule causes the fluorescent proteins to come in close proximity with each other, increasing the likelihood of FRET and resulting in a measurable shift from cyan to yellow fluorescence. This molecular approach for calcium imaging has also been shown to be applicable in a video-rate two-photon system (Fan et al., 1999), and its implication for plant–microbe interactions (Heath, 2000) has been discussed.

15.6.4 Fluorescent Proteins

Fluorescent protein applications are in effect limitless and have repeatedly demonstrated their utility with filamentous fungi and their allies (Lorang et al., 2001; Bourett et al., 2002; Czymmek et al., 2002) and with yeast (Cormack, 1998). Because fluorescent proteins are genetically coded, they may be expressed in any genetically tractable fungus via molecular approaches as a fusion protein or reporter molecule. Their utility for fungi with semipermeable and chemically variable cell wall biochemistry can be readily appreciated. This approach is rapidly becoming the method of choice for visualizing gene expression, subcellular structures, and protein localization. From the first report of GFP expression in a filamentous fungus, *Ustilago maydis* (Spellig et al., 1996), others have used confocal microscopy with fluorescent proteins to monitor gene expression, protein localization, and mitosis in *Aspergillus nidulans* (Fernandez-Abalos et al., 1998), septum formation (Fox et al., 2002), protein secretion (Gordon et al., 2000), nuclear and cytoplasmic tubulin distribution (Ovechkina et al., 2003), reef coral fluorescent proteins (Bourett et al., 2002), and pathogenicity genes (DeZwaan et al., 1999), and to monitor plant disease (Czymmek et al., 2002), to name just a few. One could readily envision a library of fungi having specific promoters, organelles, or structural proteins (e.g., endoplasmic reticulum — Figure 15.7, nucleus, mitochondria, microtubules, microfilaments) tagged either singly with a fluorescent protein or as multicolor transformants. Such a library could serve as a powerful resource for exploring how these various structures or reporters are involved in growth and development, drug treatment, and environmental stimuli. Fluorescent protein technology has become even more interesting of late with the development of photoactivatable GFP (PA-GFP) and kindling fluorescent protein (KFP). More specifically, they provide new opportunities for selectively inducing localized fluorescence and following subsequent dynamic changes of activated molecules. The PA-GFP molecule can be photoconverted from a nonfluorescent species to a fluorescent one via UV excitation (Patterson and Lippincott-Schwartz, 2002). The 405-nm diode laser used with confocal microscopes, or pulsed multiphoton laser excitation is ideally suited for this task. For example, a population of GFP-tagged vesicles incorporated into the Spitzenkorper could be photoactivated and their fate documented to determine if they were secreted or destined for distal regions of the hypha. Cotransformation with a nonactivating visible fluorescent protein (e.g., RFP) in conjunction with PA-GFP would permit the total population vs. the activated population of vesicles to be visualized simultaneously. As a supporting technology, fluorescent proteins are likely to have the largest impact on fungal imaging in the foreseeable future and will be the predominant tool for tagging and monitoring cellular events in fungi amenable to transformation.

15.6.5 Four-Dimensional Imaging

One area that is less established and clearly underutilized is the collection of three-dimensional data sets over time, or four-dimensional imaging. When used in conjunction with fluorescence confocal/multiphoton imaging, a wealth of potential information can be derived. Four-dimensional microscopy typically involves acquiring three-dimensional data sets at a single location at consecutive time points (Thomas et al., 1996). (See Figure 15.7c–e.) As with any technique, a number of factors must be considered to optimize four-

dimensional experiments, including control of environmental conditions for potentially prolonged periods, minimizing photobleaching and phototoxic effects from excessive exposure to the laser light source, avoiding motion artifacts, and the storage, management, and analysis of sizable data sets. Data analysis is best undertaken with dedicated software for extraction of critical and relevant information (Thomas et al., 1996). With the appropriate software, features may be tracked, quantified, and measured in two, three, or four dimensions. For example, four-dimensional tracking was applied in *Arabidopsis* root hairs to determine the extent and nature of nuclear migration (Van Bruaene et al., 2003). One could use similar methods to track specific fungal structures, such as the spindle pole body during nuclear division. Another detailed four-dimensional confocal investigation of *Dictyostelium* used GFP variants as cell-type reporters and a combination of actin-GFP fusion protein and Cell Tracker Green to analyze cell sorting during slug tip formation (Zimmermann and Siegert, 1998). The authors provided excellent insight on useful methods for data analysis and presentation. Four-dimensional confocal imaging by Czymmek et al. (2002) demonstrated the utility of this approach during fungal invasion and colonization of a plant host by tagging the fungal pathogen with a cytoplasmically expressed fluorescent protein. They were able to document appressorial penetration and subsequent infection hyphae ramification at individual infection sites over a 24-h series. Four-dimensional data acquisition has also been employed to continuously monitor *Fusarium oxysporum* root invasion over 3 days during vascular wilt disease in *Arabidopsis* (Fogg, Kang, and Czymmek, unpublished data).

The ability to collect multiple user-defined positions in a single experiment significantly increases efficiency of data collection. Four-dimensional data collected in combination with a motorized x-y stage would allow opportunities to visit multiple locations, automatically focus (Bürglin, 2000), collect z-stacks, and move back to the original point or any number of defined positions repetitively (Czirok et al., 2002). However, this is best applied for processes having relatively slow dynamics (i.e., changes on the order of minutes rather than seconds), since the time involved in movement of hardware components could cause significant gaps in information. There is little doubt, even with these few studies, that documentation and analysis of the multidimensional growth patterns of fungi would greatly benefit using a four-dimensional approach.

15.6.6 Multiphoton Imaging of Fungi

Even though a relative newcomer in the field of microscopy, several mycological investigators have been able to utilize the advantages of multiphoton imaging. The first published multiphoton reports for fungi monitored nuclear behavior in germ tubes of the AM fungus *Gigaspora* using DAPI for *in vivo* time-lapse imaging (Bago et al., 1998a, 1998b). Bago et al. (2002a, 2002b) later described the use of multiphoton for *in vivo* time-lapse imaging of Nile Red (with 840-nm excitation)-stained lipid bodies to record the size, distribution, and velocities during their movement toward the growing tip. Czymmek et al. (2002) showed a three-dimensional reconstruction of EGFP-labeled hyphae after glutaraldehyde fixation of *Magnaporthe grisea*-infected barley using multiphoton microscopy (870-nm excitation) and noted improved imaging deeper within infected plant tissue. In a review, Hyde (1998) discussed the advantages of multiphoton microscopy for improved laser penetration, reduced damage in living cells, and its small excitation volume when using the popular UV-excitable calcium indicator Indo-1 for imaging Ca^{2+} dynamics in fungi. More recently, Hyde et al. (2003) used multiphoton microscopy for excitation of the UV-excitable vacuolar dye CMAC when attempting to find vital stains that could withstand the aldehyde-free freeze-substitution process. In a recent study of biofilms with mixed populations of bacterial species, two-photon was compared with confocal microscopy and

shown to be superior when measuring pH gradients (Vroom et al., 1999). Newport Green, a complexing agent that becomes fluorescent when binding the heavy metals zinc and nickel, was imaged with confocal microcopy to investigate these metals in natural biofilms and activated sludge (Wuertz et al., 2000a, 2000b). Microbial biofilms that include fungal species would likely be suitable for similar investigations using multiphoton imaging. In the majority of these cases, the utility of multiphoton microscopy was related to its ability to image living cells, without harmful effects, deep into thick tissues (Figure 15.4) and dyes traditionally excited with UV using NIR excitation. These applications are fundamental to the core strengths of fluorescence-based technologies, and the expanded use of multiphoton imaging for mycological research will further increase their usefulness.

15.6.7 Plant–Fungal Interactions

Fungal pathogens and symbionts are well suited to benefit from the advantages of confocal imaging due to inherent sample thickness from associations with host tissue (Czymmek et al., 1994). A large body of cytology-based reports of plant–fungal interactions exists in the literature, and contemporary approaches have been recently reviewed (Hardham and Mitchell, 1998; Heath, 2000; Gold et al., 2001; Howard, 2001). Although confocal and multiphoton microscopy are the preferred methods for imaging plant–fungal interactions, it is not always a straightforward task to image deep into whole-mount samples. In fact, this is one of the greatest impediments to live cell imaging of leaf mesophyll because it can cause significant changes in the refractive index as light travels through this region: a complex of alternating thick plant cell walls, aqueous cellular contents, and intermittent air pockets. This degradation in image quality was given as the reason for restricting imaging to only epidermal cells of *Arabidopsis* leaves when visualizing subcellular reorganization of GFP-tagged tubulin, actin, endoplasmic reticulum, and Golgi in response to the oomycetes *Peronospora* and *Phytophthora* (Takemoto et al., 2003). Effects of this optical phenomenon are problematic with live tissue, but can be greatly reduced by multiphoton imaging of fixed tissue (Czymmek et al., 2002). Another approach has been to use ethanol clearing in conjunction with chloral hydrate and mounting in Hoyer's medium (Duncan and Howard, 2000). Samples optically cleared in this way, treated with an FITC-conjugated lectin, and imaged with confocal microscopy proved to be an excellent way to document disease progression of the leaf blotch pathogen *Mycosphaerella graminicola*. A similar preparation strategy was used with cleared, aniline blue-stained *Arabidopsis* embryos to determine the three-dimensional architecture and cell fate (Bougourd et al., 2000). Although this method provides superb resolution and optical sectioning qualities, it is likely to be restricted to primarily fluorescent probes and dyes whose binding sites are not altered or extracted by the harsh clearing methods. Even without chloral hydrate clearing, tetramethyl rhodamine isothiocyanate (TRITC)-conjugated lectin labeling methods were used to create excellent three-dimensional stereographic reconstructions of *Uromyces* infection hyphae in susceptible and resistant pea cultivars (Harding et al., 1999).

Despite optical limitations, confocal microscopy has provided enhanced *in planta* views of the infection process. Mellersh and Heath (2001) used confocal microscopy to image the dynamics of cytoplasmic Hechtian strands in plasmolyzed peas loaded with fluorescein diacetate and noted a correlation between increased plasma membrane–cell wall adhesion sites and decreased likelihood of penetration by rust fungi. A fluorescent protein fusion in the form of a pathogenesis-related protein Pth11-GFP localized to the plasma membrane and vacuoles (aniline blue staining and confocal microscopy of fixed cells were also included in this study) (DeZwaan et al., 1999). Constitutively expressed EGFP in the cytoplasm was used to follow penetration and subsequent invasion of *Magnaporthe grisea* in the leaf of a susceptible barley cultivar in four dimensions (Czymmek

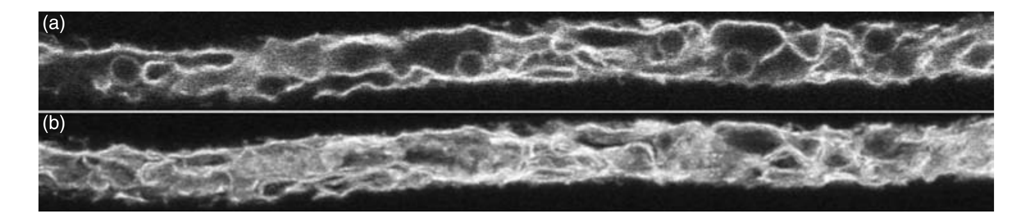


Figure 15.8 *In vivo* confocal images of the fluorescent protein AcGFP targeted to the endoplasmic reticulum via the signal peptide KDEL of *Fusarium*. A single optical section demonstrated ER-GFP localization in a living nonapical cell (a). Small circular rings represent nuclear envelope-associated labeling. A three-dimensional maximum intensity projection (b) of four optical z-sections acquired at 1-μm intervals, including image a, which demonstrated the sheet-like nature of the endoplasmic reticulum. (Images provided courtesy of K. Czymmek, T. Bourett, J. Sweigard, and R. Howard.)

et al., 2002). The authors also were able to demonstrate the extracellular penetration of mesophyll cells *in vivo*. Lagopodi et al. (2002) used a cytoplasmic expressed fluorescent protein to document aspects of root pathogenesis of tomato inoculated with *Fusarium oxysporum* in live samples that were grown and inoculated in soil and subsequently washed before imaging with confocal microscopy. There are few studies of plant–fungal pathogenic interactions of roots imaged with confocal microscopy, but they can be especially informative when combined with GFP technology and three-dimensional (Figure 15.8b) and four-dimensional analysis. This relatively unexplored area will likely experience rapid growth in the next several years.

Because a large majority of plants have beneficial symbiotic mycorrhizal affiliations, understanding details of these interactions, including nutrient exchange, could provide valuable clues for fostering these advantageous relationships. An increasing body of literature confirms the benefits of confocal and multiphoton microscopy for imaging mycorrhizal interactions. For example, Melville et al. (1998) used a series of xanthene dyes to stain LR-White sections of mycorrhiza and determined that sulforhodamine G resulted in the most useful staining pattern for imaging up to 100 μm deep into the sections with negligible autofluorescence from plant material. In frozen, cryosectioned materials, acid fuchsin was preferred for quantifying surface area and volume of mycorrhizal structures (Dickson and Kolesik, 1999). The study took full advantage of the confocal microscope's ability to generate three-dimensional data for quantification. This sectioning approach would also appear compatible with other fluorescent probes and proteins when thicker tissues prove problematic for dye penetration and image quality. Acid fuchsin staining of *Glomus* and *Gigaspora* in a tomato mutant had greatly reduced AM colonization as determined by surface area and volume of mycorrhizal structures (Barker et al., 1998).

Several investigators also have used immunofluorescence techniques as a tool for imaging the cytoskeletons of both mychorrhizal host and fungus, as recently reviewed by Timonen and Peterson (2002). For example, microtubules in *Asparagus* (Matsabura et al., 1999) and *Medicago*, with wheat germ agglutinin staining (to visualize fungal walls), were used to demonstrate changes in host tubulin in response to fungal ingress (Blancaflor et al., 2001). It is not surprising that other plant structures would also be affected, as was the case with GFP-labeled tobacco plastids in mycorrhizal root associations of acid fuchsin-stained *Glomus intraradices* (Fester et al., 2001) and the rearrangements of the cytoskeleton and endomembrane system during pathogenic interactions (Takemoto et al., 2003).

In vivo imaging of mycorrhizal associations is far less common but very possible. For example, unstained autofluorescent AM structures of *Glomus*-infected rye grass were

highly amenable to confocal microscopy due to the small size and transparency of the plant roots (Vierheilig et al., 1999). *In vivo* imaging of tobacco phloem loaded with the fluorescent probes cSNARF and CFDA showed that cSNARF labeled all fungal structures, while CFDA was restricted to only the cells with highly developed arbuscules (Vierheilig et al., 2001). Currently, due to the complex obligate nature of mycorrhizal fungi, stable transformation is problematic, and thus fluorescent proteins cannot be used as tools to explore mycorrhizal associations from the fungal perspective. Once suitable transformation methods are developed, one might expect that confocal/multiphoton imaging in this particular area of fungal biology will realize even greater significance, as it is increasingly being used to study pathogenic interactions.

15.7 CONCLUSIONS

Modern microscopy has already granted unprecedented views and details of important fungal cytological events, and the prospects for continued development of supporting technologies leave little doubt that discoveries of far greater import will be forthcoming. The optical sectioning techniques afforded by multiphoton and confocal microscopy, as described herein, arguably enhance both the quality and quantity of data over the majority of conventional fluorescence-based imaging techniques. Parallel development of a multitude of technologies, including but not limited to improved computer, hardware, software, optics, fluorescence chemistry, and molecular biology, will drive and sustain incremental improvements in this field. A greater understanding of confocal/multiphoton system capabilities and increased cooperation among the various disciplines are encouraged and will foster the exploitation of these powerful approaches to benefit mycological research.

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16

Enzymatic Activities of Mycelia in Mycorrhizal Fungal Communities

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16.1 INTRODUCTION

Knowledge of fungal biology is still poorly integrated into general ecology theory. Fungal mycelia play a pivotal role in many ecosystem processes, but a better understanding of the ways in which they shape plant communities and ecosystems will not be possible without an improved understanding of the spatial organization of fungal mycelia, including microscale interactions with substrates and other organisms. Fungal mycelia interact with and modify their environment at a range of spatial scales, influencing microscale interactions between cells and molecules, meso- and macroscale effects on organisms and growth substrates, and ultimately whole plant communities and ecosystems. Hyphal tips of fungi produce a wide spectrum of molecules, including organic acids, polysaccharides, hydrophobins, antibiotics, siderophores, and enzymes. These molecules have a diverse array of functions and mediate cellular recognition, morphogenesis, antibiosis, pathogenesis, stress tolerance, degradation, and nutrient mobilization, as well as symbiotic associations. Abundant release of lytic enzymes into the environment is a central ability of fungi that they share with various prokaryotes, but which distinguishes them from other eukaryotic organisms. During evolution, fungi have tended to live as endobionts of other organisms in parasitic or mutualistic relationships. In mycorrhizal symbiosis, these two fungal characteristics may interact to enable the plant hosts to benefit from the superior ability of their fungal associates to mobilize nutrients from complex, organic substrates.

This chapter will review recent studies of the potential of mycorrhizal fungi to produce extracellular enzymes and thereby utilize complex organic resources in the soil. Many studies have focused on the enzyme production of mycelium growing in axenic culture. In order to evaluate the potential to produce extracellular enzymes under more realistic conditions, enzyme activities have also been studied in soil microcosms containing mycorrhizal plants. More recently, molecular techniques have been used to explore the genetic potential of mycorrhizal fungi to produce different enzymes. The application of molecular tools in the field is now necessary to demonstrate the expression of this potential under natural conditions.

16.2 EVOLUTIONARY ASPECTS OF ENZYME PRODUCTION

The evolutionary history of fungi began with the split from the animals about 1 billion years ago. The ancestral fungi developed in aquatic environments, possibly as parasites on animal hosts (Berbee and Taylor, 2001). Chytridomycetes, a group that diverged from other fungi at an early evolutionary stage and are thought to have conserved many traits of their ancestors, are often parasites on invertebrates or algae, but several are saprotrophs that degrade organic polymers such as cellulose, keratin, and chitin (Powell, 1993). The major evolutionary radiation of fungi took place during the Palaeozoic era along with the evolution of terrestrial plants, and the vast majority of fungi today are closely associated with plants as parasites, symbionts, or saprotrophs (Berbee and Taylor, 2001). When vascular plants emerged (about 460 million years ago), the terrestrial environment was most likely already colonized by fungi, and even some of the oldest plant fossils show evidence of mycorrhizal associations with Glomalean fungi (Pirozynski and Dalpé, 1989). The majority of the early terrestrial fungi may have lived as endophytes, either symbionts or parasites, but as a large fraction of the potential energy in plant tissues is bound up in cellulose and hemicellulose in the cell walls, one would expect a development of plant endophytes toward necrotrophy or saprotrophy.

Cellulolytic enzymes have developed in bacteria, fungi, plants, and even some animals, and the ability to degrade cellulose is spread among all major systematic groups of fungi (Lynd et al., 2002). In more recalcitrant plant tissues, such as wood, the cellulose fibers are protected from degradation by a more or less extensive coating of polyphenolic lignin compounds. The degradation of lignin is thought to be an endothermic process that involves an energetic cost to the degrading organism (Kirk and Farrell, 1987), but complete or partial removal of the lignin is necessary for the cellulolytic enzymes to be able to act on their substrates. Plants with lignified tissues were present already in the Devonian era about 400 million years ago (Robinson, 1996), but most fossil records of wood-rotting fungi are much younger (Taylor and Osborn, 1996). The radiation of homobasidiomycetes, the fungal group in which a major fraction of the wood-rotting fungi is found, has been estimated to have taken place about 200 million years ago (Berbee and Taylor, 1993). There may thus have been a gap of more than 100 million years between the occurrence of woody plants and the evolution of fungi with the enzymatic capacity to degrade lignocellulose. This enzymatic lag phase on the evolutionary scale has been proposed as an explanation for the buildup of fossil carbon during the Carboniferous era, as well as the reduction in atmospheric CO_2 concentration that occurred during this period (Robinson, 1996). Eventually, intricate enzyme systems, involving various oxidizing enzymes, developed that allow many contemporary fungi, basidiomycetes in particular, to degrade polyphenolic lignin and humus compounds. These enzymes provide them with an almost exclusive ability to degrade the woody plant tissues, in which 80% of the global organic carbon pool is sequestered (Rayner and Boddy, 1988).

Most likely, other trophic strategies of basidiomycetes developed within lineages with wood-degrading ancestors (Malloch, 1987). Coniferous trees within the *Pinaceae* emerged about 120 million years ago, and it is likely that ectomycorrhizal (ECM) symbiosis evolved together with the host plants (Brundrett, 2002). The ability to form ectomycorrhizal symbiosis is widely spread among the different basidiomycetous lineages, and several ascomycetes may also form ectomycorrhiza. The ectomycorrhizal habit seems also to have been lost on several occasions — many saprotrophic taxa seem to be derived from mycorrhizal ancestors (Hibbett et al., 2000). Mycorrhizal symbiosis with plants renders fungi less dependent on cellulolytic enzymes for their acquisition of carbohydrates. The relative demand for other nutritional resources such as nitrogen and phosphorus is, however, likely to be higher in mycorrhizal fungi than in saprotrophic fungi because they are connected to tree hosts that are large nutritional sinks. Extracellular nitrogen- and phosphorus-mobilizing enzymes, such as proteases, chitinases, phosphatases, and nucleases, are widespread throughout the fungal kingdom. Although the ectomycorrhizal fungi are dispersed within the evolutionary tree of some of the most capable producers of extracellular enzymes, their ability to mobilize nutrients from complex organic sources and to support their plant hosts with nutrients assimilated in organic form has been recognized only relatively recently (see Lindahl et al., 2002; Read and Perez-Moreno, 2003 and references within). The polyphenol-oxidizing enzyme systems that enable many basidiomycetes and some ascomycetes to degrade lignocellulose may play an important role in the mobilization of nitrogen and phosphorus, since these elements tend to be tightly bound in polyphenolic complexes in many forest ecosystems (Handley, 1961).

16.3 ENZYMATIC POTENTIALS EXPLORED IN AXENIC CULTURES

Belowground communities of ectomycorrhizal and, to a lesser extent, ericoid mycorrhizal fungi have been extensively studied in recent years, and we now have a reasonable knowledge of the structure and dynamics of these communities under a range of environmental conditions (Horton and Bruns, 2001; Perotto et al., 2002). In contrast, our functional understanding of ectomycorrhizal and ericoid mycorrhizal fungal communities remains fairly limited, and most of what we know is based on activities of isolated fungi in axenic culture or studies of host plants inoculated with the fungi in gnotobiotic or nonsterile conditions.

Screening for ecologically relevant enzyme activities in axenic culture has been important in establishing that many ectomycorrhizal and ericoid mycorrhizal fungi have the potential to produce enzymes that may contribute to degradation of components of soil organic matter and mobilize nitrogen and phosphorus from simple organic substrates (reviewed in Leake and Read, 1997; Read and Perez-Moreno, 2003). Production of extracellular proteases has been demonstrated for several ectomycorrhizal and ericoid mycorrhizal taxa (Leake and Read, 1990; Zhu et al., 1990; Tibbett et al., 1999; Nehls et al., 2001b), while growth in media containing simple proteins implies that a range of other taxa produce similar activities (e.g., Abuzinadah and Read, 1986; Chen et al., 1999; Taylor et al., 2000; Sawyer et al., 2003a). Isolates of the ericoid mycorrhizal fungi *Hymenoscyphus ericae* and *Oidiodendron griseum* (= *O. maius*) were further found to be capable of accessing nitrogen in protein–polyphenol complexes, while only certain ectomycorrhizal taxa showed a very limited ability to use complexed protein (Bending and Read, 1996b). This is suggested to reflect production of polyphenol-oxidizing enzymes by the ericoid mycorrhizal fungi and greater resistance of the ericoid mycorrhizal proteases to inactivation

by polyphenols (Bending and Read, 1996a,b, 1997), and may reflect differences in nitrogen acquisition strategies between the two groups of fungi.

Abuzinadah and Read (1986) classified ectomycorrhizal fungal taxa as protein fungi or nonprotein fungi on the basis of their relative proteolytic abilities as evidenced by growth of single isolates on protein as a sole nitrogen source in axenic culture. Although the classification remains widely used as the basis for inferences of relative ecological functioning, it is clear that ectomycorrhizal fungi display considerable intraspecific variation (see Cairney, 1999). *Amanita muscaria*, for example, was classified by Abuzinadah and Read (1986) as a protein fungus; however, some genotypes of this fungus do not appear to fit this description (Sawyer et al., 2003c). Similarly, Taylor et al. (2000) reported that an isolate of *Paxillus involutus*, classified as a protein fungus by Abuzinadah and Read (1986), showed no proteolytic capability. Classifications based on activities of single isolates in axenic culture are thus likely to be misleading and may lead to erroneous inferences of ecological functioning.

Production of extracellular chitinase activities by isolates of *H. ericae* and several ectomycorrhizal fungi has also been demonstrated in axenic culture (Mitchell et al., 1992; Hodge et al., 1995). The ectomycorrhizal fungi produce both exo- and endo-acting activities (Hodge et al., 1995), and for *H. ericae*, at least, chitinase can facilitate access to nitrogen in fungal necromass (Kerley and Read, 1997).

A range of ectomycorrhizal and ericoid mycorrhizal fungi has been shown to produce extracellular and cell surface-bound phosphomonoesterase activities in axenic culture (e.g., Mousain and Salsac, 1986; Straker and Mitchell, 1986; Antibus et al., 1992). Notwithstanding that hydrolysis of phytates in soil may be limited by their relatively low solubility (Joner et al., 2000b; Tibbett, 2002), production of these enzymes is commonly thought to reflect an ability of the fungi to derive phosphorus from inositol phosphate in soil (Read and Perez-Moreno, 2003). Evidence for use of phosphorus from inositol phosphate by mycorrhizal fungi in soil, however, remains sparse, with different studies yielding apparently contradictory results (Tibbett, 2002). Certain ectomycorrhizal and ericoid mycorrhizal fungi also produce phosphodiesterase activity in axenic culture that, along with phosphomonoesterase, may facilitate access to phosphorus in nucleic acids (Ho, 1987; Leake and Miles, 1996; Myers and Leake, 1996). Because several other ectomycorrhizal and ericoid mycorrhizal fungi can use DNA as a phosphorus source in axenic culture (e.g., Griffiths and Caldwell, 1992; Chen et al., 1999; Sawyer et al., 2003b), it seems likely that phosphodiesterase production is relatively widespread among these fungi. The importance of phosphodiesterases in the phosphorus nutrition of mycorrhizal fungi in soil, however, remains somewhat speculative.

The axenic culture approach has also been useful in demonstrating that the external environment may strongly influence production and activities of the enzymes. Phosphomonoesterase production by certain ectomycorrhizal fungi in axenic culture is, for example, inversely related to orthophosphate availability in the external environment (e.g., Kroehler et al., 1988). Extracellular protease activity in axenic cultures of *H. ericae* remains significant at pH 1.5, which is considered an adaptation to nitrogen acquisition in the acidic mor humus heathland soil environment (Leake and Read, 1990). Similarly, optimal activity of ectomycorrhizal fungal proteases generally occurs at ca. pH 3.0, which is within the broad bulk soil pH range for soils inhabited by many ectomycorrhizal tree taxa (Zhu et al., 1994; Tibbett et al., 1999; Nehls et al., 2001b). A second protease with a broad optimum of pH 3 to 5.5 is also produced by the ectomycorrhizal basidiomycete *Amanita muscaria* and is excreted only when the external pH is in the range 5.4 to 6.3 (Nehls et al., 2001b). Because the pH of soil bacterial biofilms is often within this range, it has been suggested that this protease may be important in ectomycorrhizal fungus-mediated mobilization of bacteria-derived proteins (Nehls et al., 2001b). Increased production and activity of phosphomonoesterase and protease

in Arctic isolates of ectomycorrhizal *Hebeloma* spp. grown at low temperature in axenic culture may reflect a habitat-related adaptation to nutrient acquisition (Tibbett et al., 1998, 1999). Another example may be the resistance of *H. ericae* phosphomonoesterase activity to the high concentrations of Fe^{2+} and Al^{3+} typically found in acid soils dominated by ericoid mycorrhiza (Shaw and Read, 1989). Axenic culture work has further been useful in identifying that some ectomycorrhizal, and ericoid mycorrhizal fungi may produce enzyme activities that may degrade components of dead plant cell walls (reviewed in Leake and Read, 1997; Cairney and Burke, 1994, 1998b; Read and Perez-Moreno, 2003).

It is important to emphasize, however, that the axenic culture work has employed only a limited array of isolates of a handful of easily culturable ectomycorrhizal fungal taxa that produce conspicuous fruiting structures in the field. We thus remain largely ignorant of the enzymological potential of taxa that are difficult or impossible to culture, such as the ectomycorrhizal taxa *Cortinarius* and *Russula*, or resupinate basidiomycete taxa that form inconspicuous fruiting structures (Jones et al., 2003). Similarly, most enzyme-related investigations of ericoid mycorrhizal fungi have been performed on a few isolates of *H. ericae* or some other ericoid mycorrhizal fungi from northern hemisphere habitats (Cairney and Ashford, 2002). Our current perspective of enzymes in ecological functioning of mycorrhizal fungi is thus limited to only a handful of taxa and is compounded by the fact that certain purported enzyme activities in ectomycorrhizal and ericoid mycorrhizal fungi probably represent activities of other related enzymes or chemical reactions in culture media (e.g., Cairney and Burke, 1998a; Burke and Cairney, 2002). The ecological relevance of screening for enzymes that are active against simple substrates in an artificial environment and in the absence of a plant host and other microorganisms is increasingly questioned (Read and Perez-Moreno, 2003). Because the soil environment may significantly reduce substrate–enzyme interactions, activities measured in axenic cultures in the laboratory should be considered potential activities (Tate, 2002) and do not necessarily reflect activity of the enzymes in the soil environment. On the other hand, enzyme expression could be repressed in pure cultures, e.g., due to the presence of glucose in the growth medium. Nehls et al. (2001a) suggested the glucose concentration to be important in regulating differential gene expression between the Hartig net within the mycorrhizal roots and the extraradical mycelium. According to this hypothesis, enzymes active within the roots are induced in response to glucose, which is usually present at relatively high levels within the roots, and enzymes active in the extraradical mycelium are repressed in response to glucose, which is rarely encountered in the soil environment. An additional problem with axenic studies is that of catabolite repression due to the absence of a plant host, which acts as a sink to prevent accumulation of nutrients.

Because arbuscular mycorrhizal fungi have not yet been grown in axenic culture in the absence of plant roots, less is known regarding production of extracellular enzymes by these fungi than for the other mycorrhizal types considered here. There is only limited evidence that arbuscular mycorrhizal fungi may contribute to decomposition processes in soil (Hodge et al., 2001). From what is currently known, arbuscular mycorrhizal fungi appear to have limited abilities to produce cellulolytic, hemicellulolytic, and pectinolytic activities, and these are regarded as having a localized role in establishment of the symbiosis (García-Garrido et al., 2002). In contrast, experiments with monoxenic cultures of roots infected by arbuscular mycorrhizal fungi have provided compelling evidence that the fungi produce phosphomonoesterase activities that can hydrolyze organic phosphates and facilitate phosphorus transfer to the host roots (Joner et al., 2000a; Koide and Kabir, 2000). While some experiments suggest that arbuscular mycorrhizal infection can enhance host phosphorus acquisition from organic phosphates (e.g., Feng et al., 2003), the extent to which the activities expressed in soil are those of arbuscular mycorrhizal fungi or

associated microorganisms remains unclear. The observations that much of the phosphomonoesterase may be associated with fungal cell walls (Joner and Johansen, 2000; van Aarle et al., 2002) and that extracellular phosphomonoesterase activity in arbuscular mycorrhizal fungi appears not to be induced by either phosphorus limitation or the presence of the organic phosphorus substrate (Joner et al., 2000a; Olsson et al., 2002) raise questions regarding the involvement of the enzymes in phosphorus mobilization in soil. Histochemical staining has revealed that fungus-derived phosphomonoesterase activity exists in the vicinity of mycorrhizal hyphae in soil (Feng et al., 2002). Whether this activity reflects a secreted extracellular enzyme or leakage of an enzyme involved in intracellular metabolism remains unclear (Joner et al., 2000b).

Molecular screening for genes that encode ecologically relevant enzymes offers an alternative to biochemical screening and, importantly, can be used to screen fungi that have not been isolated into culture. Chambers et al. (1999) used polymerase chain reaction (PCR) screening to identify a manganese peroxidase gene in the ectomycorrhizal Atheliaceae taxon *Tylospora fibrillosa* (Burt) Donk. Presence of genes does not necessarily mean that they are expressed by the fungi, and despite having conducted reverse transcriptase (RT)-PCR investigation of manganese peroxidase expression in *T. fibrillosa* under a broad range of axenic and gnotobiotic culture conditions, no evidence for expression of this gene has so far been obtained (D.M. Chen and J.W.G. Cairney, unpublished data). Thus, while some ectomycorrhizal and ericoid mycorrhizal fungi can bring about partial degradation of lignin and lignin model compounds in axenic culture (Trojanowski et al., 1984; Haselwandter et al., 1990), these molecular data appear to support the hypothesis that this may be mediated by means other than lignin or manganese peroxidase production. It is possible that carbohydrate oxidase activities in the fungi release extracellular H_2O_2 that reacts with iron in the environment to produce hydroxyl radicals that can initiate partial combustion of lignin (Burke and Cairney, 1998; Cairney and Burke, 1998a). Such a mechanism is thought to contribute to lignin degradation by brown-rot fungi (e.g., Backa et al., 1992), but its ecological relevance in mycorrhizal fungi requires clarification.

Laccase is thought to contribute to degradation of lignin by some saprotrophic fungi (Hatakka, 1994), and the molecular screening approach has been used to demonstrate the presence of laccase-like genes in certain ectomycorrhizal fungi, notably Russulales and Atheliaceae taxa (Chen et al., 2003). In this case, however, RT-PCR indicated that the genes are expressed under a range of conditions in axenic culture and so may be functionally important. Nehls et al. (2001b) also used RT-PCR, to show not only that a gene for an extracellular protease in *Amanita muscaria* is expressed in axenic culture, but also that expression is regulated by pH, carbon availability, and to a lesser extent, nitrogen availability. In a wide range of ectomycorrhizal fungal taxa, Lindahl and Taylor (2004) demonstrated the presence of genes coding for N-acetylhexosaminidases. These enzymes take part in the degradation of chitin — a potentially important source of nitrogen in acidic forest soils. Clearly, many of the limitations associated with interpretation of biochemical screening in axenic cultures also apply to molecular studies in pure culture. Identification of candidate genes in axenic culture, however, is an essential step in development of these methods to investigate their expression in intact systems in soil.

16.4 ENZYME ACTIVITIES IN INTACT MYCORRHIZAL ASSOCIATIONS

Intact mycorrhizal associations include a plant host, which supplies carbon to the fungal symbiont, obviating the need for exogenous carbohydrates, and in addition acts as a sink

for nutrients absorbed by the fungal mycelium. These features, together with the possibility of using more natural model substrates under gnotobiotic or nonsterile conditions, permit the development of a more natural, differentiated mycelium.

Enzyme activities of roots can be examined by incubating intact samples in liquid media containing chromogenic or fluorogenic enzyme substrates. This method has been employed on several occasions to estimate the production of phosphatases by mycorrhizal roots. Both monophosphoesterase activity and diphosphoesterase activity, as well as inositol phosphatase activity, can be found on the surface of ectomycorrhizal roots (Antibus et al., 1997; Colpaert et al., 1997). Conn and Dighton (2000) found the monophosphoesterase levels on ectomycorrhizal roots to be at least twice as high as on noncolonized roots, and Colpaert et al. (1997) found inositol phosphatase activities of ectomycorrhizal *Pinus* roots to be two to four times has high as those of noncolonized roots. Plant roots, however, frequently produce extracellular phosphatases unaided by mycorrhizal symbionts, and noncolonized roots of laboratory-cultivated seedlings have sometimes been found to be associated with higher monophosphoesterase activities than ectomycorrhizal roots (Cumming, 1993, 1996; Firsching and Claassen, 1996). Higher monophosphoesterase activities have been found on ectomycorrhizal roots collected from within relatively phosphorus poor substrates than on roots collected from within substrates richer in phosphorus (Alexander and Hardy, 1981; Colpaert et al., 1997; Conn and Dighton, 2000). Furthermore, Moorhead and Linkins (1997) found ectomycorrhizal *Betula nana* roots to respond to elevated atmospheric CO_2 levels with increases in the phosphatase activity. The phosphatase activity of mycorrhizal roots thus seems to be regulated according to phosphorus availability and demand.

Although many studies have been devoted to the enzyme activities of the mycorrhizal root tips only, many mycorrhizal species form more or less extensive extraradical mycelia. In many species with extensive extraradical mycelium, the ectomycorrhizal mantles are impregnated by water-repelling substances that limit the potential for interaction with organic substrates in the soil solution (Unestam and Sun, 1995). In these species, release of enzymes and nutrient uptake are likely to be restricted to the extraradical mycelium.

In experiments using laboratory microcosms with natural substrates, the activities of enzymes involved in mobilization of nutrient from organic matter have been related to the presence of an extraradical mycelium of ectomycorrhizal fungi. In nonsterile peat microcosms, Bending and Read (1995b) examined enzyme activities in patches of degraded litter colonized by an extraradical mycelium of the ectomycorrhizal fungus *Paxillus involutus* growing from the roots of *Betula* seedlings. Colpaert and van Laere (1996) and Colpaert et al. (1997) used perlite containers with mycorrhizal *Pinus sylvestris* seedlings to study the production of extracellular enzymes by the extraradical mycelium of the ectomycorrhizal fungi *Thelephora terrestris* and *Suillus bovinus.* In one of the studies, mycorrhizal mycelium was allowed to colonize nonsterilized *Fagus* leaves, and for comparison, activities were also measured in beech leaves inoculated with the saprotrophic litter fungus *Lepista nuda* (Colpaert and van Laere, 1996). Timonen and Sen (1998) studied enzyme activities in extracts of various fungal tissues collected from microcosms with *P. sylvestris* growing in association with either *S. bovinus* or *P. involutus* in nonsterilized forest floor material.

Bending and Read (1995b) and Colpaert and van Laere (1996) found that the protease activity was two to four times higher in litter substrates colonized by extraradical mycelium of the three studied ectomycorrhizal fungi than in uncolonized litter. The protease activities were, however, low compared with those measured in litter colonized by the saprotrophic *L. nuda*.

Significant phosphatase activity was found to be associated with the presence of extraradical mycelium of *S. bovinus* and *T. terrestris* (Colpaert and van Laere, 1996;

Colpaert et al., 1997; Timonen and Sen, 1998). Colonization of beech leaves by *S. bovinus* increased the phosphatase activity to the same extent as colonization by the saprotrophic fungus. However, only low levels of phosphatase activity were found to be associated with the extraradical mycelium of *P. involutus* (Bending and Read, 1995b; Timonen and Sen, 1998). No isositol phosphatase activity could be dectected in perlite colonized by *S. bovinus* or *T. terrestris* mycelium (Colpaert et al., 1997).

Phenol-oxidizing enzyme activities were found in association with the extraradical mycelium of both *S. bovinus* and *P. involutus*, but not *T. terrestris* (Bending and Read, 1995b; Colpaert and van Laere, 1996; Timonen and Sen, 1998). In beech leaves colonized by *S. bovinus*, the phenol-oxidizing activity was half of the activity found in leaves colonized by *L. nuda*. No activity of peroxidases could be related to the occurrence of ectomycorrhizal mycelium (Bending and Read, 1995b; Timonen and Sen, 1998). Whereas the cellulase activity was relatively high in beech leaves colonized by the saprotrophic *L. nuda*, no detectable cellulase activity was found in litter colonized by the ectomycorrhizal fungi (Colpaert and van Laere, 1996).

Enzyme activities have also been assayed in the field in soil samples from localized mycelial patches of ectomycorrhizal *Hysterangium* and *Gauteria* species that form dense mycelial mats, easily distinguished from the surrounding noncolonized soil (Griffiths and Caldwell, 1992). Protease and phosphatase activities were often found to be twice as high in these mats compared with the surrounding soil. Peroxidase activities were one to two orders of magnitude higher within the mats than outside. Interestingly, the cellulase activity was also found to be higher within these patches of intense mycorrhizal colonization.

Many ectomycorrhizal fungi are known to produce lytic enzymes when growing in axenic cultures, and it is thus enticing to ascribe the increased enzyme activities in natural substrates colonized by ectomycorrhizal mycelium to direct enzyme production by the ectomycorrhizal fungi themselves. However, caution should be exercised because these experiments usually use nonsterilized organic substrates that contain a wide range of other microorganisms. These microorganisms may be stimulated by the presence of mycorrhizal mycelium and produce lytic enzymes themselves. For example, bacteria may live in close association with ectomycorrhizal hyphae, possibly metabolizing carbohydrates exuded by the hyphae (Garbaye, 1994; Sun et al., 1999). Furthermore, the mycorrhizal mycelium itself may act as a substrate for saprotrophic fungi (Lindahl et al., 2001) and induce these to produce enzymes employed in degradation of mycorrhizal hyphae.

16.5 ECOLOGICAL SIGNIFICANCE AND METHODOLOGICAL CHALLENGES

Although the first demonstration of the ability of laboratory-cultivated ectomycorrhizal fungi to take up amino acids and transfer nitrogen to their host plants was as early as 1953 (Melin and Nilsson, 1953), progress in demonstrating the significance of organic nitrogen utilization in the field has been slow, and many terrestrial ecologists still ignore the possible effects of mycorrhizal fungi. Much evidence has subsequently been gained from laboratory microcosms containing natural substrates, but field experiments are more difficult to perform. In one field experiment, Näsholm et al. (1998) demonstrated that boreal forest plants could take up intact glycine. As the roots and mycelia were not spatially partitioned, it was not possible to draw conclusions about the role of mycorrhizal fungi in the uptake. This experiment demonstrated the uptake of a relatively simple organic compound of low molecular weight. Further studies are now needed to investigate the production and activity of enzymes that may degrade more complex organic polymers.

Convincing evidence that mycorrhizal fungi produce extracellular enzymes that enable them to mobilize nutrients from complex organic polymers in soil would have a major impact on our views of the functioning of terrestrial ecosystems and would involve a further expansion of the concept of plant-available nutrients. A major fraction of the nitrogen in forest soils is incorporated into polymers of amino acids or amino sugars and requires the activities of proteases and chitinases, respectively, to become available for assimilation. Most of the soil phosphorus is also organically bound, often in the form of inositol phosphates, and can only be taken up after mobilization by phosphatases or phytases. Furthermore, organic nutrient sources in forest soils are often incorporated into highly recalcitrant polyphenolic complexes (Handley, 1961). Polyphenol-degrading enzymes such as polyphenol oxidases, peroxidases, and laccases may therefore be a prerequisite for the action of other degrading enzymes.

A central problem in firmly establishing the occurrence of enzymatic production by mycorrhizal fungi in field samples is the need to distinguish mycorrhizal activity from that of plant roots and saprotrophic fungi. Fractionation of enzyme extracts into different isoenzymes enabled Timonen and Sen (1998) to separate enzymes of plant origin from fungal enzymes. To be able to discriminate between enzymes produced by ectomycorrhizal fungi and closely related saprotrophic basidiomycetes, isoenzymes must be analyzed on an amino acid level, something that may be accomplished using mass spectroscopy. Expression of genes for degradative enzymes can also be studied at the transcriptional level, where the occurrence of mRNAs of mycorrhizal origin might be used as an indicator of enzyme production by mycorrhizal fungi. Although the identification and sequencing of genes coding for enzymes involved in cellulose and lignin degradation has reached a relatively advanced stage (Martinez, 2002), exploration of the enzymes that basidiomycetes may employ to degrade other complex organic substances has only just started (e.g., Nehls et al., 2001b; Lindahl and Taylor, 2004). An increasing understanding of lytic enzyme systems at the molecular level will open up new possibilities to explore the origin of extracellular enzymes in the field.

Approaches that combine *in situ* molecular identification of ECM fungi with radio-labeled substrates and microautoradiography (Lee et al., 1999) or that combine molecular identification and stable isotope approaches (Radajewski et al., 2000) may ultimately prove useful in this context. While these methods will require considerable development before application to questions of mycorrhizal functioning, the latter, “stable-isotope probing” technique appears particularly promising. By adding substrates that are labeled with a stable isotope such as ^{13}C to soil, DNA of the microorganisms that use the substrate will become enriched with the isotope and so can be separated by density gradient centrifugation and identified by PCR following cloning or a similar method (Morris et al., 2002; Radajewski et al., 2002).

Without the capacity to produce their own enzymes to degrade organic polymers, the mycorrhizal plant–fungus associations would be dependent on the degrading activities of other microorganisms to provide assimilable compounds. According to established nutrient cycling models, nutrients are provided in inorganic form by the mineralizing activities of saprotrophic soil organisms. The growth and activities of saprotrophic soil microorganisms are generally believed to be limited by a shortage of carbohydrates rather than nitrogen and phosphorus. According to this view, the saprotrophs would therefore not compete with the plants for nutrients, but instead use organic nitrogen- and phosphorus-containing substrates as sources of energy and carbon, leaving inorganic nutrients as an unwanted by-product for the plants to take up. Recent tracer isotope studies have, however, demonstrated a high potential of soil microorganisms to retain added inorganic nitrogen, particularly in acidic, temperate forest ecosystems, and saprotrophic fungi colonizing

cellulose-rich litter are often strong sinks for nitrogen and phosphorus (Lindahl et al., 2002 and references therein). Many observations thus suggest that plants in ecosystems with low nutrient availability have to compete with saprotrophic microorganisms for organic forms of nutrients rather than depend on them for the supply of inorganic nutrients (Kaye and Hart, 1997; Lindahl et al., 2002).

Progress made in understanding the role of mycorrhizal fungi in the mobilization of N and P from natural substrates has been comprehensively reviewed by Read and Perez-Moreno (2003). The ability of ectomycorrhizal fungus–plant associations to mobilize nutrients from natural mixed organic substrates, in competition with saprotrophic organisms, has been demonstrated in experiments using microcosms (Bending and Read, 1995a). Mobilization of nutrients from intact plant tissues has been shown to take place at a low rate only (Colpaert and van Tichelen, 1996), while nutrient mobilization from tracer isotope-labeled fungal mycelium may be relatively rapid (Andersson et al., 1997, Lindahl et al., 1999). This difference may in part be a reflection of the spectrum of degradative enzymes possessed by ectomycorrhizal fungi, but it also reflects important qualitative differences between plant and microbial litter, pointed out by Leake and Read (1997). Plant litter, while occurring in larger amounts than microbial litter, is relatively nutrient poor due to reabsorption of nutrients prior to leaf abscission, and the remaining nutrients are usually incorporated into recalcitrant polyphenolic complexes. Microbial necromass has a relatively high surface area-to-volume ratio, making it more susceptible to enzymatic degradation. Recent studies have demonstrated the mycorrhizal mobilization of nutrients from a range of substrates, such as pollen (Perez-Moreno and Read, 2001a), nematodes (Perez-Moreno and Read, 2001b) and collembola (Klironomos and Hart, 2001).

While many mycorrhizal fungi also seem to possess the enzymatic potential to degrade polyphenolic compounds, Read and Perez-Moreno (2003) point out that there is little to indicate that these fungi can compete with wood-rotting saprotrophs in large woody debris during the early stages of wood decomposition. However, mycorrhizal fungi are commonly present during the later stages of wood decomposition (Nordén et al., 1999; Tedersoo et al., 2003), following the depletion of cellulose when significant amounts of nitrogen and phosphorus may be available in the form of fungal necromass. Fungal lignin-degrading enzymes may also be used to degrade nitrogen-containing humic compounds (Steffen et al., 2002), and ectomycorrhizal fungi, as well as the ericoid symbionts, may potentially play important roles in humus degradation. Högberg and Högberg (2002) observed a 45% reduction in the amounts of dissolved organic carbon (DOC) in the forest floor, in response to tree girdling. Girdling prevents the flux of carbohydrates to the roots and is likely to drastically decrease the abundance of mycorrhizal mycelium in the soil. DOC in temperate forest soil is mainly constituted by products of lignin degradation (Zech and Guggenberger, 1996), and the reduction in DOC might be explained by a reduction in the activities of humic-degrading enzymes associated with the loss of mycorrhizal mycelium.

Studies of the ability of arbuscular mycorrhizal fungi to mobilize organic nutrients have so far been limited to investigations of a few model species growing under agricultural conditions and have been largely focused on phosphatase activity. The potential of the arbuscular mycorrhizal fungus *Glomus hoi* to increase plant acquisition of nitrogen derived from organic substrates has been demonstrated by Hodge et al. (2001). However, direct mobilization of nitrogen from organic polymers has so far not been found. This may be due to the fact that most arbuscular mycorrhizal fungi studied have been isolated from environments rich in inorganic nitrogen. Arbuscular mycorrhizal plants exist in a range of environments, including those dominated by ectomycorrhizal plants, and may need to compete for available nitrogen. Studies of arbuscular mycorrhizal associations from these

environments may provide more data that reveal the full range of enzymatic capabilities possessed by these symbionts.

The diversity of ectomycorrhizal fungal communities is known to be high (Horton and Bruns, 2001), and the increasing application of molecular methods to identify arbuscular mycorrhizal fungi suggests that their diversity is higher than previously assumed. There is, therefore, a high potential for variation in enzymatic capacity between species, but also within species (Cairney, 1999). Studies of ectomycorrhizal communities along gradients of nitrogen deposition have been carried out both at a local level (Lilleskov et al., 2002) and on a trans-European level (Taylor et al., 2000). These studies have shown that species characteristic of environments with high nitrogen deposition have a reduced proteolytic capacity compared with those from communities growing in environments that are poor in inorganic nitrogen.

Degradation of polymeric substrates requires considerable investment both in the form of biomass required to colonize the substrate and in the form of enzymes required for its degradation. These investments can be protected by reducing the activity of other microorganisms in the vicinity of their hyphae through antagonistic interactions (Boddy, 2000). Production of antibiotic compounds in close proximity to the sites of enzyme release and substrate degradation will minimize losses to competing microorganisms. Production of wall-bound enzymes or release of enzymes into polysaccharide slime matrices surrounding the hyphal tips may also restrict losses to competing microoganisms (Colpaert et al., 1997). In environments with low concentrations of readily assimilable nutrients, competition for nutrients is likely to be intense (Lindahl et al., 2002). The tight spatial coupling of enzyme production and product assimilation, coupled with territorial behavior (Boddy, 2000), may make it necessary for all fungi, including mycorrhizal fungi, to produce their own enzymes, in order to gain access to readily assimilable forms of nutrients (Figure 16.1).

Although the enzymatic activity of mycorrhizal fungi may reduce the dependence of their host plants on the activities of the general decomposer community, this does not preclude the possibility that mycorrhizal fungi make use of the enzymatic capability of specific microbial populations closely associated with their hyphae (Garbaye, 1994; Sun et al., 1999). Many fungal mycelia may support a bacterial flora on the surface of their hyphae that is specifically adapted to withstand the particular antibiotic environment of their fungal host. Sun et al. (1999) discussed the possibility that ectomycorrhizal mycelia might condition the environment around their hyphal tips by exudation of water and carbohydrates, facilitating bacterial growth as well as providing an interface for interaction with substrates. In this microspatial environment, enzymes of bacterial origin could facilitate the release of assimilable compounds in close proximity to the hyphae, obviating the need for production of fungal enzymes (Figure 16.1). In microcosm studies of interactions between ectomycorrhizal fungi and meta-toluate-degrading bacteria, Sarand et al. (2000) demonstrated a mutually beneficial effect of the fungal symbiont and its associated bacteria on their ability to grow in contaminated soil.

16.6 CONCLUSIONS

Although the capacity to degrade organic polymers is not always easy to evaluate in simple laboratory experiments, the capacity of ectomycorrhizal, ericoid, and even arbuscular mycorrhizal fungi to mobilize nutrients from organic polymers with different degrees of complexity has probably been underestimated.

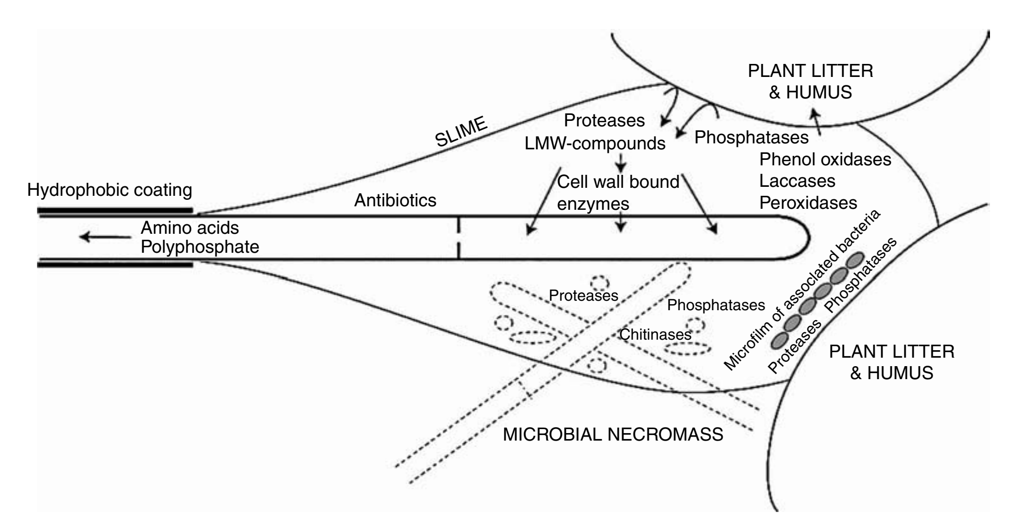


Figure 16.1 Schematic picture showing hypothetical interactions between a fungal hyphal tip and complex organic substrates during mobilization of nutrients in soil. The hyphal tip produces a slime matrix that acts as an interface to the substrates. By releasing enzymes into this matrix rather than directly into the soil solution, a high enzymatic activity may be maintained close to the hyphal tip. Furthermore, competition for assimilable compounds released into the slime from degrading substrates may be reduced through the release of antibiotics. Polyphenolic substrates may be preconditioned by oxidizing enzymes, in order to enable proteases and phosphatases to release low molecular weight (LMW) compounds. These compounds may be further processed by enzymes anchored within the cell wall, before they are taken up by the hypha. A specific bacterial community, adapted to the fungal antibiotics, may contribute to the enzymatic degradation.

The conventional view that mycorrhizal plants are totally dependent on the mineralizing activity of the decomposer community is clearly inadequate, but alternative theories, which invoke a larger degree of degradative activity on the part of mycorrhizal fungi themselves, differ in the degree of enzymatic competence attributed to the mycorrhizal and saprotrophic components.

A fundamental challenge that remains is to be able to perform realistic analyses of the true enzymatic potential using *in situ* measurements in the field. This will require the continued development of molecular tools as well as their application under ecologically realistic conditions.

The increasing taxonomic resolution with which we are now able to study fungal communities has contributed to the growing realization that there is probably a large potential for functional diversity. In addition, the mycorrhizal habit is distributed among several different evolutionary clades and care should, therefore, be exercised in generalizing about functional properties on the basis of isolated observations.

Even though awareness of the ecological importance of mycorrhizal fungi has increased, most textbooks and articles on nutrient cycling still fail to take the enzymatic potential of these fungi into account. Wider recognition of the ability of many mycorrhizal fungi to mobilize nutrients from complex organic sources is a necesssary step in the further development of nutrient cycling models, particularly in ecosystems with low nutrient availability.

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17

Fungal Enzymes at the Community Scale

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17.1 INTRODUCTION

Though they lack the metabolic diversity of bacteria, fungi make large contributions to the global carbon cycle. Hyphal organization combines the ability to assimilate nutrients along a distributed network with the capacity to focus extracellular enzyme release at growing tips (Frey et al., 2000, 2003). As a result, fungi are more efficient than bacteria at colonizing and breaking down large detritial particles. Fungi also carry more genetic information. Despite these advantages, the decomposition of organic matter remains a community process. No population can express the range of enzymes needed to degrade complex substrates, such as cell walls (Osono and Takeda, 2002). It is somewhat paradoxical that decomposition is a collective enterprise while fungal community structure and succession appear to organized by competitive, often antagonistic, interactions (Gulis and Suberkropp, 2003; Mille-Lindblom and Tranvik, 2003; Thormann et al., 2003). This paradox is more striking in light of emerging evidence that heterotrophic bacteria make extensive use of signal molecules to coordinate gene expression within and between populations, a phenomenon known as quorum sensing (Manefield and Turner, 2002; Burmolle et al., 2003; Nakayama et al., 2003).

While the population interactions that regulate community composition are quite complex, emergent measures of community function often show predictable patterns in relation to environmental variables. The most integrative metabolic variables, respiration and production, are readily linked to measures of temperature, moisture, and nutrient availability, and can be directly entered into carbon budgets. Measures of community enzyme activity are more problematic. Enzymes introduced into the environment, through directed secretion or random cell lysis, are autonomous functional agents whose activity and turnover are determined by environmental conditions and myriad biogeochemical

reactions (Sinsabaugh et al., 1994). Because activities are generally assayed under conditions far abstracted from this milieu, it is difficult to directly connect results to *in situ* degradation. On the other hand, enzyme data provide comparative measures of community effort (resources) directed toward the degradation of specific classes of compounds (Sinsabaugh et al., 2002a).

Enzyme data are most often collected to compare (or profile) community actvity in space and time. The larger goals are to integrate community structure and function and to understand the mechanics of the decomposition process. The difficulty of these tasks depends on the extent to which diverse taxa use similar environmental cues and signal transduction pathways to regulate expression of particular enzyme classes. Where there is broad commonality, the potential for ecological inference is high. Thus, this chapter is organized into two sections. The first is an overview of extracellular enzyme expression by fungi; the second presents various models that have been used to link community enzyme activity with community composition and ecosystem process.

17.2 REGULATION OF EXTRACELLULAR ENZYME EXPRESSION

In an ecological context, the enzymes most often assayed are those that mediate the degradation of polymers or catalyze the mineralization of organic nitrogen and phosphorus (Sinsabaugh et al., 2002a). Because the most abundant organic compounds are recalcitrant structural polymers of plant and microbial cell walls, the enzymes that degrade cellulose, hemicellulose, lignin, and chitin receive much study (Dekker, 1985; Eriksson and Wood, 1985; Marsden and Gray, 1986; Kirk and Farrell, 1987; Eriksson et al., 1990; Higuchi, 1990; Sinsabaugh and Liptak, 1997). Aminohydrolases and phosphatases are of interest as the proximate catalysts of N and P mineralization. It bears emphasis that most of the information available on the expression and action of fungal extracellular enzymes comes from intensive studies of a small number of model systems, e.g., the cellulases of *Trichoderma* and the ligninases of *Phanerochaete*. Extrapolating this knowledge to natural communities is problematic for at least two reasons: (1) the distribution and diversity of extracellular enzyme systems across the fungal kingdom is not well known; and (2) there are synergisms, antagonisms, and biogeochemical interactions within natural systems that are likely to strongly influence community-level patterns.

The expression of extracellular enzymes consumes significant quantities of carbon, nitrogen, and energy, an investment that is lost to the cell when enzymes enter the environment. Consequently, expression is linked to environmental signals that convey the potential for generating assimilable substrate. Details vary, but the basic regulatory cycle for extracellular enzyme expression by fungi includes a constitutive loop that sustains low-level expression and an induction loop that modulates expression in response to appropriate environmental cues (Figure 17.1). The generality of this regulatory cycle provides a conceptual and mechanistic basis for drawing inferences about community resource availability from patterns of extracellular enzyme activity. Additional information about the enzyme systems most often studied at the community level is summarized below.

17.2.1 Oxygenases and Peroxidases

Fungi release oxidative enzymes into the environment to degrade aromatic and aliphatic hydrocarbons (Higuchi, 1990). The primary function of these enzymes in plant litter decomposition is to breach lignin to gain access to shielded polysaccharides (Hammel, 1997). Even white-rot basidiomycetes, which deploy the most studied and powerful oxi-

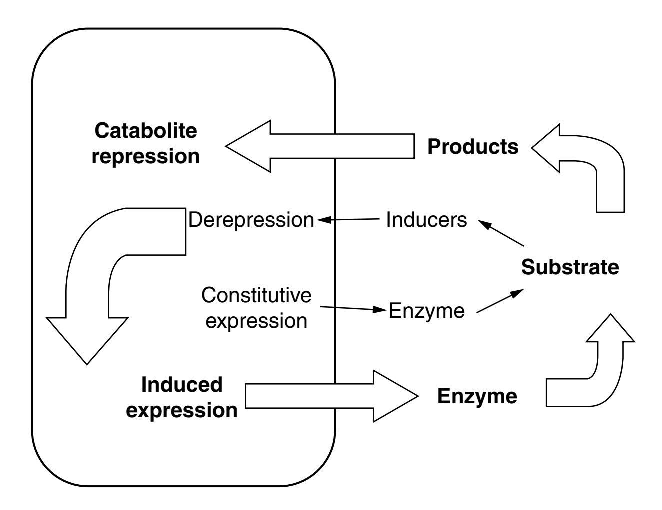


Figure 17.1 A general model for transcriptional regulation of extracellular enzyme production that links expression to environmental signals.

dative systems, cannot rely on lignin degradation products for growth. A broader, secondary function of extracellular oxidases is the breakdown of reactive aromatic molecules that can inhibit growth. Some of these molecules are by-products of lignin degradation; others are antagonistic compounds synthesized by plants and microorganisms.

The most widely distributed class of extracellular oxidases is laccases. Laccases have traditionally been defined as copper-containing enzymes that use dioxygen to oxidize diphenols. Each enzyme has four copper atoms and catalyzes four single-electron oxidations to reduce dioxygen to water. Laccases are widely distributed among fungi, bacteria, and plants. They have broad substrate specificities and variously function in both the synthesis and degradation of aromatic molecules. Until recently, it was assumed that laccases were peripheral components of lignin-degrading enzyme sysytems because their redox potentials are too low to oxidize the nonphenolic aromatic residues that form the covalent architecture of lignin. That view has changed with the discovery of white-rot fungi that degrade lignin by producing redox mediators that in effect "step up" the redox potential of laccases (Bermek et al., 1998; Li et al., 1999). Laccase production by fungi can be induced by a variety of phenolic molecules and by Cu(II) (Sethuraman et al., 1998; Palmieri et al., 2000; Scheel et al., 2000; Galhaup and Haltrich, 2001; Lo et al., 2001; Carbajo et al., 2002).

The second broad class of extracellular oxidative enzymes is peroxidases. Peroxidases are proteins that coordinate iron atoms in heme prosthetic groups. Peroxidases use hydrogen peroxide to create a reactive intermediate strong enough to withdraw electrons from nonphenolic aromatic molecules. Lignin peroxidase (LiP) first described from *Phanerochaete* (Glenn et al., 1983) is the strongest known fungal peroxidase and has been much studied (Kirk and Farrell, 1987; Hammel, 1997). Another component of the *Phanerochaete* lignin-degrading system is manganese peroxidase (MnP), first characterized by Kuwahara et al. (1984). Manganese peroxidase uses hydrogen peroxide to generate Mn(III), which chelates with acids such as glycolate or oxalate to form a diffusible oxidant. Mn(III) can also peroxidate unsaturated lipids, creating an even stronger oxidant (Bao et al., 1994). In culture studies, peroxidase production by white-rot fungi is typically induced by nitrogen starvation (Hammel, 1997).

Both LiP and MnP require a source of H_2O_2 to sustain activity. Thus, extracellular oxidases that reduce O_2 to H_2O_2 are also elements of the lignin degradation system. Glucose

oxidase, cellobiose oxidase, and aryl alcohol oxidases are examples. Another enzyme found in white-rot fungi is glyoxal oxidase, which uses small aldehydes, such as glycoaldehyde, a cleavage product of LiP, as electron donors, creating the potential for a catalytic cycle (Hammel et al., 1994).

Cellobiose dehydrogenase (CDH) is widespread among cellulose-degrading fungi (Temp and Eggert, 1999). CDH links the degradation of cellulose and lignin by oxidizing cellobiose and reducing reactive quinones. This activity may reduce the repolymerization of aromatic radicals.

17.2.2 Glycosidases

The literature on fungi and cellulose decomposition is extensive and frequently reviewed (e.g., Eriksson and Wood, 1985; Ljungdahl and Eriksson, 1985; Marsden and Gray, 1986; Eriksson et al., 1990). The complete degradation of cellulose requires three types of hydrolytic enzymes: exo-1,4-β-glucanases (cellobiohydrolases), which are capable of binding to crystalline domains and hydrolyzing cellobiose or glucose from the nonreducing ends of cellulose molecules; endo-1,4-β-glucanases, which randomly cleave glucosidic linkages along noncrystalline domains; and 1,4-β-glucosidases, which release glucose from celloligosaccharides.

For white-rot fungi, the decomposition of native cellulose is the result of synergistic interaction between exo- and endoglucanase; neither enzyme alone is able to effect significant breakdown. These glucanase components are released into the environment by growing hyphae, while β-glucosidases, which generate assimilable products, generally remain associated with the hyphal wall (Cai et al., 1999). Cellulase production, although constitutive, can be stimulated by the presence of cellulose degradation products and other monosaccharides (Morikawa et al., 1995).

Compared with lignin and cellulose, hemicellulose is labile. The largest constituents of hemicellulose are xylan and mannan. Xylan is a polymer of β-1,4-linked xyloses with 4-O-methylglucuronic acid and L-arabinose side chains (Viikari et al., 1994). Mannans are polymers of glucose and mannose, in random order, connected by β-1,4-glycosidic linkages with galactose and glucose side chains. The most extensively studied hemicellulase systems are those of the soft-rot fungi *Aspergillus* and *Trichoderma* (see reviews by Biely, 1985; Dekker, 1985; Wong et al., 1988; Eriksson et al., 1990; Duarte and Costa-Ferreira, 1994; Viikari et al., 1994). Endo-1,4-β-xylanases and endo-1,4-β-mannases depolymerize the main chains. The resulting oligosaccharides are further hydrolyzed by 1,4-β-xylosidases, 1,4-β-D-mannosidases, and 1,4-β-glucosidases. Branch chain cleavage requires α-L-arabinosidases, α-glucuronidases, and α-galactosidases. As with cellulase, hemicellulase production can be induced by various degradation products (Simão et al., 1997).

Chitin, a β-1,4 polymer of N-acetylglucosamine, is a cell wall component of most fungi. Chitinases are made by mycoparasites to gain access to hosts, and deployed by potential hosts as a defense against fungal infection (Hodge et al., 1995; El-Katatny et al., 2001). In the decomposition process, endochitinases and exochitinases decompose chitin from cell walls and arthropod exuviae into chitobiose, which is hydrolyzed to monomers by β-1,4-N-acetylglucosaminidase (Cabib, 1987; Pitson et al., 1993). Some of these enzymes may also contribute to the breakdown of peptidoglycan from bacterial cell walls.

17.2.3 N and P Metabolism

Studies with model fungi show that the expression of various extracellular peptidases and aminohydrolases is controlled by a nitrogen regulatory circuit (Marzluf, 1997). At the cellular level, this circuit is complex. At the community level, a key point is that high ammonium and glutamine availability can result in catabolic repression of extracellular

enzyme expression (Farley and Santosa, 2002), while nitrogen deprivation is likely to induce expression.

Fungi produce both acid and alkaline phosphatases, as well as enzymes commonly known as phytases that are able to hydrolyze phosphate from inositol penta- and hexaphosphates (Joner et al., 2000; Yadav and Tarafdar, 2003). Because intracellular phosphatases play a prominent role in cellular metabolism, it is difficult to determine what fraction of community activity is the result of secretion of extracellular enzymes relative to incidental release through cell lysis. However, acid phosphatases are generally assumed to be extracellular enzymes. Phosphatase activity can be induced by phosphorus limitation, and high activity relative to other metabolic or enzyme activities is often interpreted as an indicator of P limitation.

17.3 ACTIVITY PATTERNS AT COMMUNITY LEVEL

Fungi and other osmotrophs are dependent on extracellular enzymatic activity for nutrition. Yet, enzymes released into the extracellular environment, whether through regulated secretion or cell lysis, become autonomous agents. Activity and turnover are determined by physical conditions, e.g., temperature and water potential, and biogeochemical interactions, e.g., sorption and humification (Figure 17.2). Consequently, the decomposition of organic matter can be represented as a successional interaction, or loop, involving the organic matter substrate, the fungal community, and the extracellular enzyme pool (Figure 17.3). The successional loop model provides a conceptual framework for studying the dynamics of extracellular enzyme activities in relation to decomposition.

Traditionally, decomposition has been represented as a function of residual mass, litter composition (e.g., C:N ratio, lignin content), or environmental conditions (e.g.,

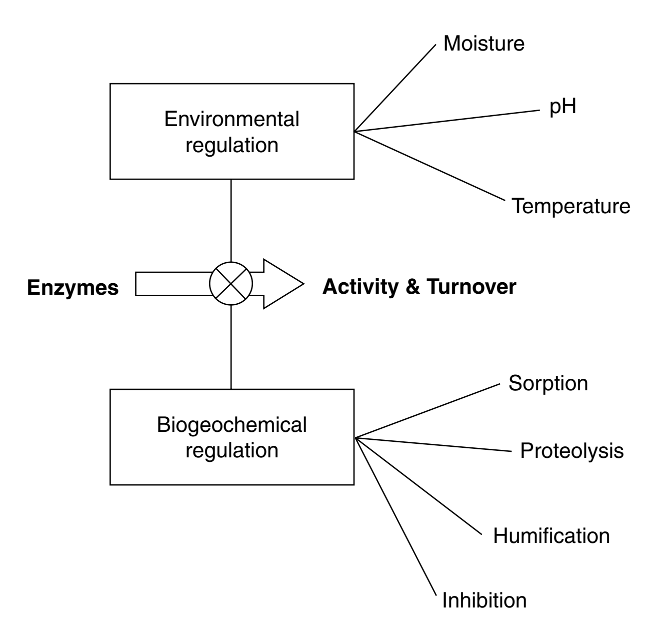


Figure 17.2 Factors and processes that control the activity and turnover of enzymes released into the environment.

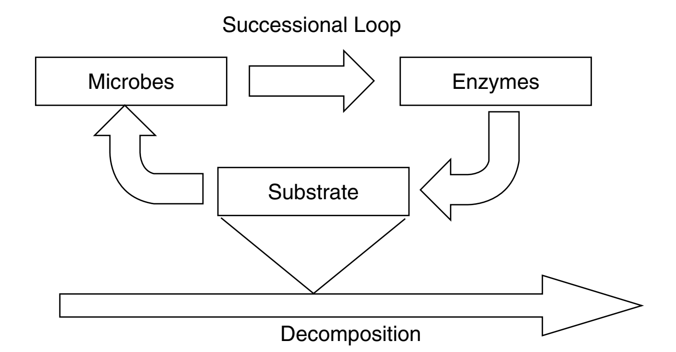


Figure 17.3 Microbial community composition and extracellular enzyme dynamics are tied to organic matter decomposition through a successional loop.

temperature, moisture). All these approaches are useful at large scales, but they become increasingly problematic at fine scales because single parameters cannot capture the complexities of the successional loop (Sinsabaugh and Moorhead, 1996; Moorhead and Sinsabaugh, 2000). Decomposition models that incorporate microbial activity have been developed, but they are difficult to evaluate because there are very few empirical studies that include information on litter composition, microbial metabolism, and enzyme activity (Sinsabaugh et al., 2002a).

Schimel and Weintraub (2003) have recently developed a simulation model that links decomposition to enzyme kinetics and uncouples the enzymatic degradation of organic matter from microbial metabolism. This approach assumes that decomposition is a nonlinear function of enzyme concentration; i.e., the rate of assimilable carbon generation per unit enzyme activity decreases with enzyme concentration because the number of effective substrate binding sites is limited (Sinsabaugh and Linkins, 1989). Restricted substrate access can lead to microbial carbon limitation even when the organic matter pool appears large. Differential turnover rates, lags, and kinetic dissonance between the extracellular enzyme pool and microbial community can also disconnect substrate generation from microbial assimilation and disconnect nitrogen acquisition from carbon supply. The model provides a mechanistic basis for a common observation: labile carbon additions, e.g., glucose, tend to increase microbial respiration, while increased N availability often promotes growth at the expense of respiration. The model clearly demonstrates that traditional C vs. N limitation dichotomies are not applicable to decomposition systems and establishes the value of the successional loop paradigm as a foundation for decomposition models.

The literature on extracellular enzyme activity (EEA) patterns in relation to litter composition, fungal dynamics, and mass loss has been recently reviewed (Sinsabaugh et al., 2002a). To minimize repetition, information from that review is presented here in summary form.

Cohorts of newly senescent litter decomposing over time show a characteristic pattern of EEA that correlates with fungal succession and changing substrate composition. Enzymes such as α-glucosidase and invertase that act on soluble saccharides appear early, then decline markedly. β-glucosidase activity also tends to be high during the early stages of decomposition, but because of its role in cellulolysis, activity remains significant throughout the process. The activities of the other cellulases (exo- and endoglucanases) increase more slowly and peak near median mass loss. As accessible cellulose disappears, the ratio of endoglucanase to exoglucanase tends to increase. Laccase activity tends to

increase with lignin–humus content of litter, but activities can also be relatively high early in decomposition for litters with a high content of soluble secondary compounds. For heavily humified material, peroxidase activities typically exceed those of laccase. These trends occur in both aquatic and terrestrial systems. However, environmental fluctuations that substantially alter temperature, moisture, and nutrient availability may affect enzyme activities and obscure or overwhelm patterns linked to litter quality (Figure 17.2).

For particular litter types, various enzyme activities have been correlated with mass loss rates (Jackson et al., 1995; Sinsabaugh and Findlay, 1995). To compare enzymatic decomposition across litter types, turnover activities can be calculated from models of mass loss as a function of cumulative enzyme activity, analogous to traditional models that describe litter decay over time as a first-order function of residual mass. Cumulative enzyme activity is computed by integrating the area under a curve of enzyme activity vs. time; the results can be expressed in units of activity-days (or simply as total moles of substrate converted). A linear regression, natural log (cumulative activity) vs. time, generates a first-order rate constant called the apparent enzymatic efficiency. Inverting this rate constant produces an estimate of litter turnover expressed in units of activity-days. Turnover activities provide a basis for comparison across sites, treatments, and litter types, and provide relative indices of how much enzymic effort and what type of enzymic effort a microbial community exerted to decompose a cohort of litter (Sinsabaugh et al., 2002a).

When litter or soil from a particular site is repeatedly sampled and assayed for EEA, there are often strong correlations among various activities (Larsen et al., 2002; Saiya-Cork et al., 2002; Sinsabaugh et al., 2003). These correlation patterns presumably reflect underlying congruence in carbon, nitrogen, and phosphorus flux, as well as commonalities in the cellular and environmental regulation of enzyme activities. As a broad generalization, factor analysis of EEA profiles tends to condense cellulase and other glycosidase activities into a single variable. Laccase and peroxidase activities also tend to covary and condense into a single variable. N-acquiring activities, e.g., aminohydrolase and peptidase, tend to group; phosphatase activity may vary independently of other activities. These observations underlie a resource allocation model proposed by Sinsabaugh and Moorhead (1994, 1996). The model is based on the assumption that many taxa use similar regulatory pathways to tie extracellular enzyme expression to substrate availability. When integrated on a community scale, this regulatory convergence should resemble an optimal resource allocation strategy for maximizing microbial productivity (Figure 17.4). The model predicts trade-offs among efforts to get carbon, nitrogen, and phosphorus from organic sources.

There have been few attempts to link community composition or biodiversity with enzymatic process. Maire et al. (1999) examined the relationships among soil respiration, microbial diversity (using phospholipid fatty acid analysis), and the activities of xylanase, laminarinase, phosphatase, urease, and chitinase. They found a correspondence between functional diversity and structural diversity, both peaking in spring. Maamri et al. (1998, 1999) found that differences in breakdown rates and cellulolytic activity between permanent and temporary stream sites were associated with differences in fungal diversity and bacterial biomass. But Raviraja et al. (1998) and Zak et al. (1995) found no relationship between fungal diversity, enzyme activities, and mass loss.

Zak et al. (2003) examined the interactions between microbial community diversity and activity and plant diversity in soils beneath experimental plots that contained 1 to 16 species. Microbial biomass, respiration, fungal abundance, and N mineralization increased with plant diversity, but the changes appeared to be responses to increased plant productivity, rather than plant diversity. However, increasing microbial N mineralization apparently contributed to the positive relationship between plant diversity and productivity.

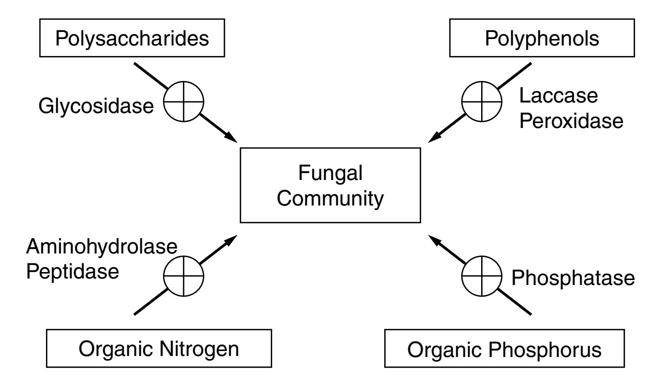


Figure 17.4 A resource allocation model that accounts for community-scale patterns in extracellular enzyme activities. Commonalities in the cellular and environmental regulation of enzyme activity, and the nutrient requirements for growth may generate correlation patterns among extracellular enzyme activities that reflect the availability or demand for particular nutrients.

Perhaps the best documented example of the significance of shifting from an ecosystem to a microbially based decomposition paradigm is the case of atmospheric nitrogen deposition. The effect of exogenous N availability on organic matter decomposition has been studied extensively (Fog, 1988; Berg and Matzner, 1997; Hobbie, 2000). Inorganic N addition often increases mass loss rates for litter that has a relatively low content of lignin, tannin, and other secondary compounds. Mass loss rates for lignified or humified material do not increase with added N and often decline. Recently these observations have been linked to changes in the distribution of EEA: cellulase and other glycosidase activities associated with litter and soil tend to increase in response to N additions, while the activities of oxidative enzymes needed to break down secondary compounds and humus tend to decline (Carreiro et al., 2000; Saiya-Cork et al., 2002; Michel and Matzner, 2003; DeForest et al., 2004; Gallo et al., 2004). This N-driven redirection of microbial EEA appears to reduce the efficiency of decomposition (mass loss per unit activity) even in situations where mass loss rates increase (Sinsabaugh et al., 2002b). At present, the best single predictor of the effect of N deposition on decomposition rates is the phenol oxidase (laccase) response: when activity increases, so does turnover rate; when activity decreases, turnover slows (Carreiro et al., 2000; Waldrop et al., 2004).

In the context of Schimel and Weintraub's (2003) model, N control of cellulase activity appears to be physiological and ecological: N addition to an N-limited environment is likely to increase microbial growth and thereby increase the constitutive production of cellulase. The significance of the higher cellulase activity for carbon flow to decomposers (and for mass loss rates) will depend on the availability of effective substrate binding sites. In contrast, the critical N control of extracellular oxidative activity appears to lie at the transcription level.

For some white-rot basidiomycetes, it is well established that high N availability blocks expression of lignin-degrading peroxidases (Hammel, 1997). This phenomenon has often been discussed as a possible mechanism for N inhibition of decomposition (Fog, 1988; Berg and Matzer, 1997). But not all white-rot fungi exhibit this N-dependent expression, and many white-rot fungi do not produce lignin or Mn peroxidase. In addition, losses (or gains) of laccase and peroxidase activities occur in systems that vary widely in community composition (DeForest et al., 2004; Gallo et al., 2004). Although much remains to be learned, it is clear that in some instances high N availability inhibits decomposition

at the community level by uncoupling the hydrolytic and oxidative activities needed for lignocellulose degradation (Figure 17.4). The net effects for decomposition vary with substrate and microbial community composition, and these in turn affect ecosystem scale processes such as soil respiration, carbon storage, and dissolved organic matter export.

17.4 SUMMARY

Interest in the ecology of extracellular enzymes stems from their role as semiautonomous agents in fungal growth and organic matter decomposition. Questions related to these roles can be posed on several scales, and like other ecological phenomena, new relationships emerge as scale expands. At the cellular level, the interpretation of various enzyme activities is directly related to elucidating the signals and regulatory pathways that control expression (Figure 17.1). To the extent that this information is known, enzyme activities serve as a reporter of nutrient availabilities in relation to microbial metabolism. On a larger biogeochemical scale, EEA is influenced by physical factors and numerous inhibition, sorption, translocation, and denaturation processes that attenuate relationships between substrate generation and microbial assimilation, and affect the formation of humic substances and dissolved organic matter (Figure 17.2). At the microbial community scale, diversity may influence the composition of the extracellular enzyme pool and the potential for synergistic interaction among enzymes. At the same time, commonalities in nutrient requirements and regulatory pathways across taxa lead to correlative relationships within various classes of extracellular enzymes (Figure 17.4). These patterns can provide a comparative basis for identifying the principal substrates supporting community metabolism and the relative enzymatic effort expended to acquire them. On the scale of decomposition process, extracellular enzymes become part of a successional loop that ultimately results in the mineralization of detrital organic matter (Figure 17.3). At this scale, enzyme activity data serve as indicators of large-scale environmental change and provide information on the mechanisms that underlie decomposition responses to environmental disturbance.

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18

Using Isotopic Tracers to Follow Carbon and Nitrogen Cycling of Fungi

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18.1 INTRODUCTION

The difficulty of experimental manipulations, observations, and measurements in the environments inhabited by fungi has long hindered studies of fungal functioning. One promising tool to explore fungal functioning, stable isotope measurements, is increasingly used to study links between fungi and biogeochemical processes. Such measurements rely on calculating ratios of heavy to light isotopes of key biological elements (e.g., ^{13}C:^{12}C, ^{15}N:^{14}N, ^{18}O:^{16}O, ^{2}H:^{1}H, and ^{34}S:^{32}S). Studies can generally be classified into two main types: those using natural abundance levels of isotopes in fungi or fungally produced compounds and those in which compounds or substances artificially enriched in one or more of the heavy isotopes are applied as tracers and the subsequent fate of the tracer is followed into different ecosystem components, including fungi. Natural abundance and tracer studies can be used at different levels of resolution, ranging from whole organisms to specific compound classes (e.g., lipids), specific compounds (e.g., N-acetyl glucosamine), or even DNA or RNA unique to a single species. Compound-specific analyses using gas chromatography of volatile compounds linked to isotope ratio mass spectrometry have been particularly widely applied (reviewed in Boschker and Middelburg, 2002). The use of isotopic techniques has increased dramatically in the past 15 years as a result of several technological advances, including faster analyses with the widespread adoption of continuous-flow isotope ratio mass spectrometry, improvements in our ability to measure hydrogen and oxygen isotope ratios, and steady increases in the quality and variety of compound-specific measurements possible. In the following chapter, I focus primarily on using natural abundance measurements of carbon and nitrogen isotopes to assess fungal

functioning in ecosystems, with some discussion of tracer experiments. Because interpreting natural abundance measurements requires an understanding of how isotopic differences are created, I also discuss the mechanisms creating nitrogen and carbon isotope patterns. Recent useful reviews on natural abundance measurements include Adams and Grierson (2001), Evans (2001), Werner and Schmidt (2002), and Hayes (2002).

Although fungi display great plasticity in their patterns of resource acquisition, fungi are functionally separated by their primary carbon source into mycorrhizal, saprotrophic, and parasitic life history strategies, with most fungi belonging to the first two categories. Saprotrophic fungi obtain carbon from the decay of dead organic matter, whereas mycorrhizal fungi form symbioses with plants in which plant-supplied sugars are exchanged for nutrients obtained by fungi from the soil. Mycorrhizal fungi can be further divided into several types, of which the two most important are arbuscular mycorrhizal (AM) fungi (belonging to the Glomerales), symbiotic with most herbaceous plants and most tropical trees, and ectomycorrhizal fungi. Ectomycorrhizal fungi associate with many of the dominant tree families of temperate and boreal regions (e.g., Pinaceae, Betulaceae, Salicaceae, and Fagaceae), but also some families distributed in the tropics (e.g., Dipterocarpaceae and Myrtaceae, with the eucalypts of Australia being the most prominent ectomycorrhizal members of the latter family). Higher fungi in the phyla Basidiomycota and Ascomycota have been tempting targets for isotopic measurements in recent years because many produce large, conspicuous fruiting bodies that often can be identified to species. These large fruiting bodies have allowed researchers to analyze isotopes of individual species without resorting to complex techniques such as DNA- or RNA-specific analyses. Such isotopic analyses are providing interesting insights into patterns of carbon and nitrogen cycling by both ectomycorrhizal and saprotrophic fungi in these phyla.

18.2 NATURAL ABUNDANCE MEASUREMENTS

Most physical, chemical, and biochemical processes favor the initial incorporation of the lighter isotope in the product, leaving the substrate enriched in the heavy isotope. The magnitude of this isotopic fractionation differs depending on the elements involved and the specific reaction mechanism. Isotopically fractionating processes therefore result in differences in the isotopic ratios between the substrate and the product. These ratios depend on the isotopic ratio of the substrate, the proportion of substrate transformed to product, and whether the system is open or closed (Figure 18.1).

Natural abundance studies use isotopic differences among different ecosystem pools and compounds to understand the sources and fluxes of many biologically important elements. Because differences in isotopic ratios are small, they are measured using δ notation, as deviations in parts per mille (‰) from a standard ratio, according to Equation 18.1:

$$\delta^{n}X\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000 \qquad (18.1)$$

In Equation 18.1, n represents the atomic mass of the heavy isotope, X the symbol for the element of interest, and R the molar abundance of the heavy isotope divided by the light isotope (e.g., $^{13}C/^{12}C$). The isotopic standard for carbon is Vienna PeeDee Belemnite ($^{13}C/^{12}C$ = 0.0112372); for nitrogen, atmospheric N_2 ($^{15}N/^{14}N$ = 0.0036765); and for hydrogen and oxygen, Vienna Standard Mean Ocean Water (V-SMOW, D/H = 0.00015576, $^{18}O/^{16}O$ = 0.00200520) (Hoefs, 1997). Isotopic values for carbon generally range from 0‰ (carbonates) to –50‰ (methane in some systems), with most $\delta^{13}C$ values in systems dominated by plants of the C_3 photosynthetic pathway ranging from –20‰ to –35‰. In

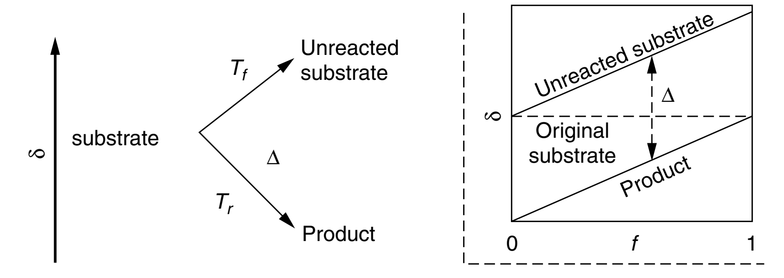


Figure 18.1 Isotopic composition of substrate and product in an open system depends on the fraction (f) of substrate transformed to the product and the isotopic fractionation (Δ) of the reaction. Isotopic patterns for product (δ_p) and substrate (δ_s) for open and closed systems are shown as a function of f, Δ, and the initial ^{15}N of substrate (δ_i). In a closed system the flux of new substrate replenishing the substrate pool is zero. (a) Open system, $\delta_s = \delta_i + f \bullet \Delta$, $\delta_p = \delta_i - (1 - f) \bullet \Delta$. (b) Closed system (Rayleigh distillation), with Δ = 10‰. $\delta_s = \delta_i - \Delta \bullet \ln(f)$, $\delta_p = \delta_s + \Delta \bullet (\ln[1 - f])/f$. The isotopic signature of the instantaneous product is also shown, which is simply $\delta_p = \delta_s - \Delta$.

systems dominated by plants of the C_4 photosynthetic pathway, values range from –10‰ to –20‰. For $\delta^{15}N$, pools in terrestrial ecosystems are usually between 20‰ and –10‰. In comparisons among samples, samples with more of the heavy isotope are commonly referred to as isotopically enriched, or heavy, and samples with less of the heavy isotope are referred to as isotopically depleted, or light. Isotopic fractionation () for reactions in open systems can be calculated based on the isotopic signature of the source and product according to Equation 18.2:

$$\Delta = (\delta^n X_{source} - \delta^n X_{product})/(1 + \delta^n X_{product}) \qquad (18.2)$$

An important distinction in interpreting isotopic patterns is whether kinetic or equilibrium isotopic effects dominate in a reaction. In kinetic isotopic effects, the light isotope invariably reacts faster than the heavy isotope, so that the resulting product is depleted in the heavy isotope relative to the substrate. Isotopic effects during irreversible reactions, such as many decarboxylations, are governed by kinetic isotopic effects. In contrast, in equilibrium reactions a back reaction from product to substrate also occurs, and therefore, the kinetic isotope effects for both the forward and back reaction must be considered. In equilibrium reactions the heavy isotope generally concentrates in the compound with stronger bonds (Bigeleisen, 1965). For example, in the equilibrium reaction between ammonia and ammonium, the ammonium ion accumulates 19 to 21‰ more ^{15}N than ammonia, and in the equilibrium reaction between CO_2 and bicarbonate, bicarbonate accumulates 8‰ more ^{13}C than CO_2. This latter reaction largely accounts for the 8‰ depletion of atmospheric CO_2 relative to marine bicarbonate.

18.2.1 Carbon Isotopes

One of the clearest patterns emerging from field studies is that fungi are enriched in ^{13}C relative to commonly measured reference materials such as wood or leaves. Saprotrophic fungi are 3 to 4‰ enriched in ^{13}C relative to woody or litter substrates (Kohzu et al., 1999; Hobbie et al., 2001; S. Trudell, unpublished data), and ectomycorrhizal fungi are enriched by 1 to 5‰ in ^{13}C relative to current-year foliage (Hobbie et al., 1999a; Högberg et al., 1999b; Kohzu et al., 1999). Because specific plant tissues or plant species often differ in ^{13}C content (Brugnoli and Farquhar, 2000), $\delta^{13}C$ measurements could be used to determine more precisely the probable carbon sources of fungi. For example, in a study in a mixed

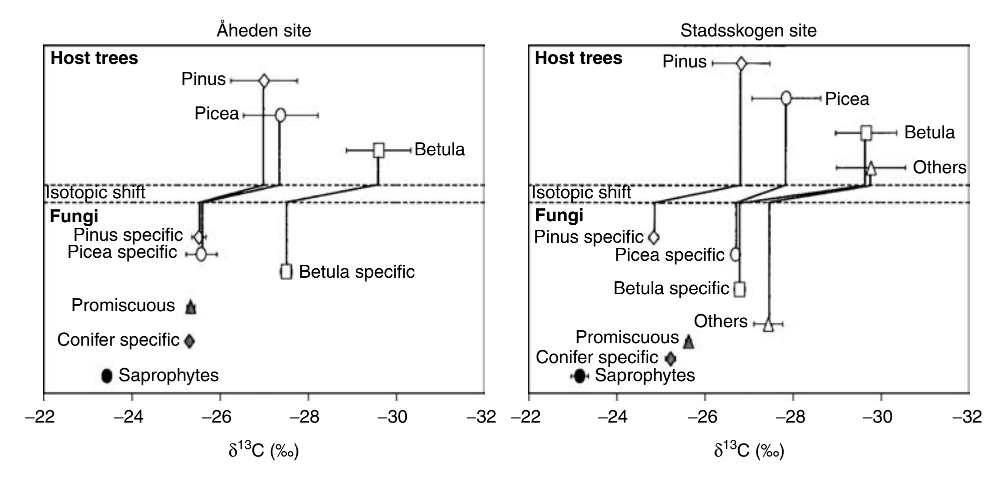


Figure 18.2 Natural abundance of ^{13}C ($\delta^{13}C$) of fungal fruit bodies and host trees of ectomycorrhizal fungi (as indicated by connecting lines) in mixed temperate forests at two sites in Sweden: Åheden and Stadsskogen. Promiscuous fungi are non-host-specific ectomycorrhizal fungi. Pine (*Pinus sylvestris*) is the dominant tree at both sites, followed in abundance by spruce (*Picea abies*) and birch (*Betula pendula*). Ectomycorrhizal fungi are arranged in groups according to their host specificity. Saprophytes are saprotrophic fungi. Bars show standard deviations of single observations. Replicates are individuals in case of host trees; in case of fungi, replicates are species. (Reproduced from Högberg et al., *Proceedings of the National Academy of Sciences, U.S.A.*, 96, 8534–8539, 1999, with permission. Copyright 1999 National Academy of Sciences, USA.)

birch–pine forest, Högberg et al. (1999b) used the fidelity between the $\delta^{13}C$ of ectomycorrhizal fungi and their putative hosts to determine that mycorrhizal fungi specific to conifer or birch hosts were 1 to 3‰ enriched in ^{13}C relative to plant host foliage, and that mycorrhizal fungi of broad host specificity primarily obtained carbon from the dominant, overstory Scots pine (Figure 18.2).

A second clear pattern is that saprotrophic fungi are several per mille enriched in ^{13}C relative to co-occurring ectomycorrhizal fungi (Figure 18.2; Hobbie et al., 1999a; Kohzu et al., 1999; Henn and Chapela, 2001). Such measurements are therefore useful in determining whether fungi of unknown life history status are mycorrhizal when assessed along with other ecosystem pools (Högberg et al., 1999b; Hobbie et al., 2001).

Recently, mycorrhizal or saprotrophic status in fungi was also verified using radiocarbon (^{14}C) measurements (Hobbie et al., 2002). The ^{14}C content of atmospheric CO_2 started to increase in the early 1950s as a result of ^{14}C created during thermonuclear testing, peaking in 1963 at about twice the background level. In this study, the declining ^{14}C content of atmospheric CO_2 since the Nuclear Test Ban Treaty of 1963 among the Soviet Union, Great Britain, and the U.S. was used to estimate the age of carbon incorporated by mycorrhizal or saprotrophic fungi at a 65-year-old *Pseudotsuga menziesii* site. Mycorrhizal fungi closely tracked current-year foliage or atmospheric CO_2 in ^{14}C content, whereas saprotrophic fungi ranged in apparent age from 3 to 50+ years. Based on the low ^{14}C content of the wood decay fungus *Pleurocybella*, it primarily assimilated "prebomb" carbon. It appeared possible to assign several taxa of unknown life history strategy to mycorrhizal or saprotrophic status based solely on ^{14}C measurements (Figure 18.3). Although radiocarbon measurements are expensive compared with stable isotope measurements (about U.S.$300 vs. U.S.$15 per sample), the technique could potentially address several issues about carbon cycling in fungi, such as incorporation of carbon

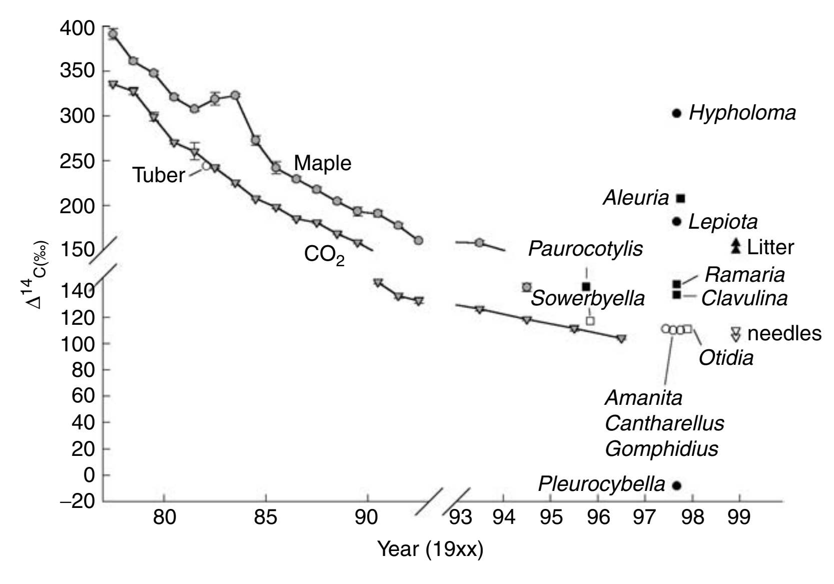


Figure 18.3 Measured values for radiocarbon ^{14}C in samples of fungi, needles, and litter. ^{14}C content is standardized at 0‰ ($\Delta^{14}C$) in 1950. Growing-season mean ^{14}C of CO_2 at Schauinsland, Germany (1977 to 1996), and of maple leaves in Quebec (1977 to 1993) are also shown ± standard error. Notice x- and y-axis scale changes of breaks at 1993 and 150‰, respectively. Symbols: empty triangles, needles; filled triangles, litter; empty circles, ectomycorrhizal fungi; empty squares, suspected ectomycorrhizal fungi; filled circles, saprotrophic fungi; filled squares, suspected saprotrophic fungi. The ectomycorrhizal fungi *Amanita*, *Cantharellus*, and *Gomphidius* and the suspected mycorrhizal fungus *Otidea* were all collected in September 1997 from Woods Creek, OR (four total). (Modified from Hobbie et al., *New Phytologist*, 156, 129–136, 2002).

derived from organic nitrogen uptake, that previously required tracer isotope experiments. Uptake of organic nitrogen should theoretically result in protein pools that reflect the ^{14}C age of soil amino acids rather than the ^{14}C age of atmospheric CO_2.

Because carbon in roots, foliage, and mycorrhizal fungi may differ in residence time, and the $\delta^{13}C$ of fixed carbon will vary depending on plant nutrient status and water stress (Brugnoli and Farquhar, 2000), ^{13}C content of different pools may partly reflect the differing environmental conditions at the time of carbon fixation. To avoid these confounding factors, culture studies under constant environmental conditions are therefore desirable. When mycorrhizal Scots pine was grown at constant internal nutrient concentrations without water stress, mycorrhizal hyphae of *Suillus luteus* and *Thelephora terrestris* were 3.2 ± 0.2‰ enriched relative to host foliage and 1.6 ± 0.1‰ enriched in ^{13}C relative to roots (E. Hobbie, unpublished data). The enrichment of fungi relative to roots was similar to a ~2‰ enrichment of cellulose relative to bulk tissue in needles and wood of *Picea abies* (Gleixner et al., 1993) and similar to a 2‰ enrichment in ^{13}C of ectomycorrhizal fungi relative to fine roots in a field study (Hobbie et al., 1999a). These studies therefore indicate that the ^{13}C enrichment of mycorrhizal fungi relative to plants probably does not reflect variations in the ^{13}C of carbon fixed at different times, but rather ^{13}C enrichment during metabolic processes.

Because plant sugars are the carbon source for both plant cellulose and mycorrhizal fungi, it would be useful to know if plant sugars are isotopically fractionated during cellulose formation or during incorporation into fungal tissues. In *Eucalyptus* plantations, xylem sugars and newly formed xylem tissue had similar ^{13}C content (Pate and Arthur,

1998). This xylem tissue is almost exclusively polymeric carbohydrates (pectic substances, hemicelluloses, and cellulose; Swift, 1976). In addition, ectomycorrhizal fungi grown on sugars do not appear to fractionate against ^{13}C (Henn and Chapela, 2000; Hobbie et al., 2004). These studies and the studies discussed in the preceding paragraph suggest that ectomycorrhizal fungi, root sugars, wood cellulose, and transferred sugars should have similar ^{13}C enrichments relative to the initial $\delta^{13}C$ of fixed carbon, whereas foliage should be slightly depleted in ^{13}C relative to these pools. This therefore suggests that ^{13}C enrichment of mycorrhizal fungi relative to foliage, the usual reference material, may primarily reflect ^{13}C enrichment during transport from leaves to roots.

The $\delta^{13}C$ of fixed carbon increases with water stress (Brugnoli and Farquhar, 2000) and will therefore often vary seasonally. The $\delta^{13}C$ values of mycorrhizal fungi that fruit at different seasons could thus provide information about the residence time of fungal carbon stores. In a recent survey in Sweden, mycorrhizal fruiting bodies declined in $\delta^{13}C$ about 1‰ over a 10-week collection period from August to October (Taylor et al., 2003), suggesting that the residence time in mycorrhizal networks of carbon from which fruiting bodies are derived could be less than 10 weeks. Similar seasonal declines in ^{13}C content of mycorrhizal fruiting bodies have been observed in the northwestern U.S. (S. Trudell, unpublished data). Previous studies of mycorrhizal fruiting patterns after artificial defoliation in birch, after >90% reductions in light levels in pines, or after stem girdling also suggest that carbon used for fruiting body formation is closely tied to current photosynthate (Last et al., 1979; Lamhamedi et al., 1994; Högberg et al., 2001).

In contrast to the ^{13}C enrichment of ectomycorrhizal fungi relative to plant hosts, spores and hyphae of AM fungi are 1 to 4‰ depleted in ^{13}C relative to plant roots (Nakano et al., 1999; Staddon et al., 1999). This depletion was attributed to the high content of ^{13}C-depleted lipids in AM fungi, particularly in AM spores, as microbial lipids can be up to 10‰ depleted in ^{13}C relative to total microbial biomass (Hayes, 2002). An additional factor that could explain ^{13}C patterns in AM fungi is the extensive transport of lipids from intracellular hyphae to extracellular hyphae and spores (Pfeffer et al., 1999). Using seedlings grown heterotrophically, Luo and Sternberg (1994) demonstrated that gluconeogenesis from lipids results in cellulose 1‰ depleted in ^{13}C relative to the substrate, whereas gluconeogenesis from starch results in cellulose 1‰ enriched in ^{13}C relative to the substrate. Given that metabolic processes should be similar for growth on alkanes and lipids, the potential effects on isotopic patterns of growth on lipids vs. growth on carbohydrates could also be inferred from a culture study with the yeast *Candida lipolytica* (Zyakun, 1996). Growth on glucose resulted in similar ^{13}C content for biomass and fungal carbohydrates relative to the substrate, whereas growth on alkanes resulted in a ^{13}C depletion relative to substrate of 2.2‰ for biomass and 4.7‰ for carbohydrates. If these results also apply to AM fungi, then transport of lipids in AM fungi followed by subsequent gluconeogenesis (Pfeffer et al., 1999) could result in ^{13}C-depleted fungal carbohydrates in AM fungi relative to plant-supplied sugars and fungal lipids.

The rather large enrichment in ^{13}C of wood decay fungi relative to wood of ~3.5‰ (Table 18.1) appears to derive from (1) ^{13}C enrichment during metabolism and (2) preferential incorporation from wood of carbohydrate-derived carbon, the most ^{13}C-enriched carbon pool in plants. Studies of growth of saprotrophic fungi on synthetic, ^{14}C-labeled lignins show that fungal metabolites such as veratryl alcohol and CO_2 were ^{14}C-labeled, but fungal biomass was not (discussed in Jennings, 1995). In a detailed study of compound-specific $\delta^{13}C$ patterns, Gleixner et al. (1993) determined that both white-rot fungi (with known ligninolytic abilities) and soft-rot fungi (without ligninolytic abilities) were equally enriched in ^{13}C relative to wood cellulose (about 1.8‰). This implies little incorporation of lignin-derived carbon by either fungal type. These studies therefore imply that white-

Table 18.1 ^{13}C Enrichment (‰) of Fungi Relative to Substrate and Respired CO_2

Substrate	Organism	$\delta^{13}C_{fungi}$– $\delta^{13}C_{substrate}$ (‰)	$\delta^{13}C_{fungi}$– $\delta^{13}C_{CO2}$ (‰)	Reference
Birch wood	*Phanerochaete chrysosporium*	3.9		(8)
Wood	Decay fungi (5 taxa)	3.5 ±0.2		(1)
Fagus wood	*Trametes versicolor*	3.5 ±0.5[a]	3	(2)
Wood	Decay fungi (5 taxa)	3.5 ±0.3		(3)
Wood	Decay fungi	3.1, 1.8[b]		(4)
Wheat straw	*Panus tigrinus*	1.5		(8)
Glucose/agar[c]	*Panellus serotinus*	1.4		(5)
Fagus wood	*Hypoxylon fragiforme*	0.3		(1)
Zea leaves	*Ustilago maydis*	0.3		(1)
Sucrose[d]	Imperfect soil fungi (12 taxa)	0.3 ±0.4		(6)
Sucrose	*Marasmius androsaceus*, *Suillus granulatus*, *Cryptoporus volvatus*	0.0 ±0.2[e]	0.1[f], 0.8[g]	(7)
Glucose	*Candida lipolytica*	–0.1	–2.1	(8)
Glucose	Ectomycorrhizal fungi (10 taxa)	–0.3 ± 0.2		(5)
Glucose	*Lycoperdon perlatum*	–0.6		(5)
Glucose/agar[c]	*Leucopaxillus gentianeus*	–0.7		(5)
n-Alkanes	*Candida lipolytica*	–2.2	4.5	(8)
Glucose	*Clitocybe nebularis*	–2.8		(5)

Note: Standard errors are given when available.

[a] Efficiency of 15 to 21%.

[b] Relative to wood cellulose.

[c] Carbon derived from agar was 30% for *Panellus* and 10% for *Clitocybe*.

[d] Some incorporation of malt extract-derived sugars.

[e] Efficiency of 54 to 62%.

[f] *M. androsaceus.*

[g] *C. volvatus.*

References: (1) Ziegler (1995); (2) Kohzu et al. (1999); (3) Hobbie et al. (2001); (4) Gleixner et al. (1993); (5) Hobbie et al., 2004; (6) Hobbie et al. (2003); (7) Henn and Chapela (2000); (8) Zyakun (1996).

rot fungi do not incorporate lignin-derived carbon, but that instead their extensive ligninolytic capabilities are used solely to improve access to cellulose and other carbohydrates. Given the well-known enrichment in ^{13}C of cellulose and hemicelluloses relative to bulk wood by ~2% (Benner et al., 1987; Gleixner et al., 1993), a strong fungal preference for incorporating carbon derived from these compound classes would combine with a ^{13}C enrichment during metabolism of 1 to 2‰ to produce the observed enrichment of 3 to 4‰ in fungal biomass relative to bulk wood.

18.2.2 Causes of $\delta^{13}C$ Patterns

$\delta^{13}C$ signatures of fungi appear primarily controlled by source $\delta^{13}C$ plus variable ^{13}C enrichments during metabolism. This enrichment appears high in saprotrophic fungi feeding on wood, low in ectomycorrhizal fungi, and negative in AM fungi (that is, AM fungi

are depleted in ^{13}C relative to their sources). Examining ^{13}C patterns in fungi grown on simple sugars vs. complex substrates provides some insight into the causes of these differing enrichments (Table 18.1). In culture studies, both mycorrhizal and saprotrophic fungi are less than 1‰ enriched in ^{13}C relative to simple sugars, whereas saprotrophic fungi are commonly 1 to 3‰ enriched in ^{13}C relative to complex sources such as wood or agar–sugar mixtures (Table 18.1).

The above ^{13}C enrichment patterns on simple vs. complex carbohydrates appear to be linked to metabolic efficiency, or the proportion of supplied carbon that is transformed into biomass (Table 18.1). Fungi growing on glucose or sucrose, with an efficiency of ~60% (Lundberg et al., 2001; also calculated from Henn and Chapela, 2000), are less than 1‰ enriched in ^{13}C relative to substrate carbon, whereas fungi feeding on polymeric carbohydrates or complex substrates, with probable efficiencies of 10 to 25% (Lekkerkerk et al., 1990; also calculated from Kohzu et al., 1999), are more than 1‰ elevated in ^{13}C relative to their substrates. Metabolic processes during fungal growth on simple sugars such as glucose and sucrose probably resemble metabolic processes of mycorrhizal fungi growing symbiotically on plant-supplied glucose, and therefore similar isotopic fractionations during metabolism should also be expected.

Because an isotopic mass balance must be preserved, ^{13}C enrichment of fungi indicates that either respired CO_2 or other excreted metabolites such as organic acids should be depleted in ^{13}C relative to carbon sources. One clue potentially linking metabolic efficiency and isotopic fractionation is that high levels of incompletely oxidized compounds such as oxalate and other organic acids can be expected when carbohydrates are present in excess of growth requirements (Jennings, 1995). In other words, when insufficient nitrogen, phosphorus, or trace metals are present to support growth, then overflow metabolism of secondary compounds such as oxalate, malate, citrate, or other organic acids is favored. Such conditions should prevail during growth on wood because of its low nutrient content, and in fact, oxalate appears to play a key role in facilitating cellulose and lignin degradation (Shimada et al., 1997). The lower nutrient content of wood versus that of litter may therefore lead to lower microbial efficiency in wood decay than in litter decay, and indirectly account for the higher ^{13}C of wood decay fungi than of litter decay fungi (Kohzu et al., 1999). Although organic acids in autotrophic tissues of plants seem rather variable in ^{13}C relative to coextracted sugars or bulk carbon (Whelan et al., 1970; Raven et al., 1982; Jamin et al., 1997; Gleixner et al., 1998; Beazley et al., 2002), organic acids in heterotrophic tissues of plants (potato tubers) appear depleted in ^{13}C relative to source carbohydrates by about 3‰ (Jacobson et al., 1970; Gleixner et al., 1998), so it is plausible that excreted organic acids in fungi would also be depleted in ^{13}C relative to source carbon. However, actual measurements of ^{13}C content in oxalate or other organic acids produced by fungi are needed.

More measurements of $\delta^{13}C$ are also needed for CO_2 respired by fungi. In growth on wood, respired CO_2 was depleted in ^{13}C relative to biomass about 3 to 4‰ (Zyakun, 1996; Kohzu et al., 1999; Table 18.1), whereas in growth on 90% C_4 sucrose and 10% malt extract, respired CO_2 was only 0 to 1‰ depleted in ^{13}C relative to fungal biomass (Henn and Chapela, 2000).

Thus, high ^{13}C enrichments of fungal biomass relative to substrates may indicate production of organic acids or high respiratory losses of CO_2 triggered by nutrient limitations. For example, the high ^{13}C enrichment of *Panellus serotinus* relative to source carbon in Table 18.1 may derive from production of simple organic acids during agar degradation. Greater production of potentially ^{13}C-depleted metabolites such as organic acids by saprotrophic fungi than by mycorrhizal fungi may therefore contribute to the general ^{13}C depletion of mycorrhizal fungi relative to saprotrophic fungi.

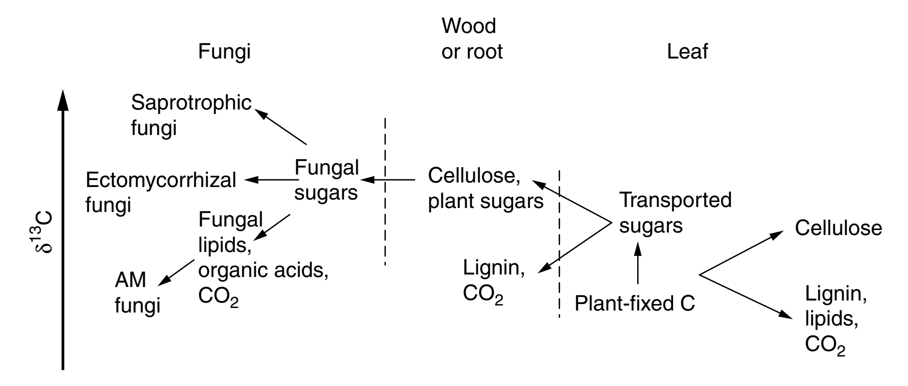


Figure 18.4 Movement and isotopic fractionation of carbon isotopes in different compounds and components of plants, mycorrhizal fungi, and saprotrophic fungi. Separate pathways are indicated for arbuscular mycorrhizal fungi and ectomycorrhizal fungi. Isotopic fractionation along nonhorizontal arrows is about 2‰.

In Figure 18.4, I propose a scheme of carbon isotope effects during carbon transfers among plants, mycorrhizal fungi, and saprotrophic fungi that accounts for current observations from field and culture studies. An important contributing factor for many isotopic patterns is the enrichment in ^{13}C of carbohydrates relative to other compound classes such as lignins and lipids. Because carbohydrate polymers are abundant in plants and possess a regular structure that is relatively amenable to enzymatic attack, they are the main carbon sources for most saprotrophic fungi; this ensures that saprotrophic fungi will be enriched in ^{13}C relative to bulk $\delta^{13}C$ of their substrates. In addition, since both plants and fungi transport carbon primarily as ^{13}C-enriched sugars (with the partial exception of AM fungi), tissues such as roots, wood, and fungal fruiting bodies become increasingly enriched in ^{13}C as the stream of labile carbohydrates becomes increasingly metabolized and a portion of the carbon is diverted to form ^{13}C-depleted lignin, lipids, and CO_2.

The precise biochemical mechanisms causing ^{13}C enrichment in carbohydrates from leaves to roots, wood, and fungi are still unknown. One strong possibility was advanced by Gleixner et al. (1993), who proposed that isotopic fractionation during triose interconversions or by aldolase during cleavage of hexose to triose controlled the enrichment in ^{13}C of fungal carbohydrates relative to cellulose. Because hexose and triose interconvert readily in plants (Hill et al., 1995), this mechanism could conceivably contribute to increases in ^{13}C content of carbohydrates with increasing distance from the source photosynthate. Because lipids are important transport compounds in AM fungi (Pfeffer et al., 1999), ^{13}C enrichment along transport pathways of these fungi appears less likely than in ectomycorrhizal fungi. Patterns of $\delta^{13}C$ in AM or ectomycorrhizal fungi relative to host-supplied sugars may accordingly reflect whether carbohydrates or lipids are the primary storage compounds used for later biosynthesis (e.g., Luo and Sternberg, 1994). And finally, relative fluxes directed to biomass formation, respiration, and organic acid production will also probably influence $\delta^{13}C$ patterns. Thus, comparing $\delta^{13}C$ patterns of sources and fungi may provide information about the relative allocation along these three pathways.

18.2.3 Nitrogen Isotopes

The close coupling between carbon and nitrogen cycling in terrestrial ecosystems (Ågren and Bosatta, 1996), the key role of mycorrhizal fungi in plant N supply, and the importance of saprotrophic fungi in decomposition and nutrient mineralization have prompted several

Table 18.2 Comparison of Response to Atmospheric N Deposition or N Fertilization, Growth on Protein N in Pure Culture, and Relative Sporocarp $\delta^{15}N$ for Ectomycorrhizal Fungal Taxa for Which Information Is Available

Taxon	Response to N Addition[a]	Growth on Protein N	Relative Sporocarp $\delta^{15}N$[b]
Lactarius theiogalus	+ + +	No	Low–medium
Paxillus involutus	+ + +	Variable	Low–high
Lactarius rufus	+ + +	Variable	Low–medium
Amanita muscaria	ND[c]	Variable[d]	Low–medium[e]
Laccaria bicolor	+ +[f]	No–poor	Low
Thelephora terrestris	+	Variable	Low[g]
Tylospora fibrillosa	= / +	Variable	ND
Cenococcum geophilum	– / =	Variable	High
Russula spp.	–[c]	Yes (1)[h]	Low
Cortinarius spp.	– –	Yes (6)[h]	Medium–high
Piloderma croceum group	– –	Yes	ND
Tricholoma inamoenum	– –	Yes	High
Suillus variegatus	– –	Yes	Medium–high
Suillus luteus	ND	Yes	Medium–high[e]
Suillus bovinus	– –[f]	Yes	High

[a] For response to fertilization: +, slightly positive; + +, positive; + + +, very positive; =, neutral; –, negative; – –, very negative.
[b] Relative $\delta^{15}N$ was determined by comparison of isotopic signatures within each study and is presented instead of absolute values because of the site effect on isotopic signatures.
[c] ND, no data.
[d] Sawyer et al. (2003).
[e] Taylor et al. (2003).
[f] Information available from studies of sporocarps only.
[g] Hyphae only; Hobbie and Colpaert (2003).
[h] Number in parentheses indicates number of species in genus tested.

Modified from Lilleskov et al. (2002).

studies of nitrogen isotope patterns in fungi and other ecosystem components in the last 10 years (e.g., Gebauer and Dietrich, 1993). In many studies, fruiting bodies of mycorrhizal fungi are enriched in ^{15}N relative to saprotrophic fungi (Hobbie et al., 1999a; Kohzu et al., 1999; Henn and Chapela, 2001; but see Gebauer and Taylor, 1999). However, $\delta^{15}N$ patterns exhibit less fidelity within and between life history strategies than $\delta^{13}C$, with differences in both source $\delta^{15}N$ and internal processing of N probably controlling the $\delta^{15}N$ of fungal N.

Mycorrhizal fungi differ greatly in $\delta^{15}N$ values depending on taxa. Lilleskov et al. (2002) compared patterns of $\delta^{15}N$ and protein use in different mycorrhizal taxa and concluded that those with greater proteolytic capabilities and diminished growth responses to additions of mineral nitrogen were generally higher in $\delta^{15}N$ than those not showing such responses. This pattern appears to hold across most genera tested (Table 18.2), which suggests that other taxa of high $\delta^{15}N$ values are likely to possess proteolytic capabilities as well.

The mechanistic basis for this correlation is still unknown. Deeper soil layers are generally enriched in ^{15}N relative to litter layers (Amundson et al., 2003), and bulk soil organic matter is generally enriched in ^{15}N relative to mineral N (Högberg, 1997). In addition, because peptide hydrolysis fractionates against ^{15}N about 4‰ (Silfer et al., 1992), free amino acids may be somewhat depleted in ^{15}N relative to bulk proteinaceous material in soils. Such comparisons have yet to be carried out. Whether exploiting different soil horizons or different forms of nitrogen influences, fungal $\delta^{15}N$ has not been satisfactorily explored, although such comparisons should be possible soon with the advent of molecular techniques to determine which mycorrhizal taxa are located at different soil depths (Rosling et al., 2003).

Differences in $\delta^{15}N$ of saprotrophic fungi may also be linked to source $\delta^{15}N$. Fruiting bodies of wood decay fungi generally have higher $\delta^{15}N$ values than litter decay fungi (Kohzu et al., 1999; Hobbie et al., 2001; S. Trudell, unpublished data). Isotopic fractionation against ^{15}N during decomposition progressively enriches the remaining organic matter in ^{15}N, with humus often 5 to 10‰ higher in $\delta^{15}N$ than undegraded plant tissues (Högberg, 1997). The $\delta^{15}N$ of saprotrophic fungi may accordingly directly reflect the enzymatic capabilities of fungi to access N in different plant and soil pools. This point must be considered with several caveats. First, metabolic processes show large isotopic fractionations in fungi, as shown by the consistent ^{15}N depletion in fungal chitin relative to protein of up to 10‰ (Taylor et al., 1997). Therefore, differences in chitin or protein concentrations in fruiting bodies could easily affect bulk $\delta^{15}N$. Second, tracer or natural abundance studies have not shown unequivocally which soil N pools are used by saprotrophic or mycorrhizal fungi. And third, soil N pools of potentially different availabilities have in general not been isotopically characterized, making it difficult to link fungal $\delta^{15}N$ patterns to source $\delta^{15}N$.

Because the dominant plants in most terrestrial ecosystems are mycorrhizal, efforts to understand what controls $\delta^{15}N$ patterns in plant cultures have recently focused on the potential role of mycorrhizal fungi. Ectomycorrhizal fungi are enriched in ^{15}N relative to host plants, indicating that mycorrhizal fungi may alter the isotopic composition of nitrogen that they take up and subsequently pass on to host plants (Högberg, 1990; Schmidt and Stewart, 1997; Hobbie et al., 1999a). Several recent culture studies have confirmed that ectomycorrhizal fungi are enriched in ^{15}N relative to host pines (Högberg et al., 1999b; Kohzu et al., 2000; Hobbie and Colpaert, 2003). In one culture study, foliar $\delta^{15}N$ and allocation of photosynthate to mycorrhizal fungi were highly and negatively correlated (Hobbie and Colpaert, 2003) (Figure 18.5), suggesting the potential use of foliar $\delta^{15}N$ measurements to indicate carbon allocation to mycorrhizal fungi.

The form in which N is transferred from ectomycorrhizal fungi to plants is unclear, but ^{15}N-depleted amino acids are probably created during transamination reactions and subsequently exported to host plants. Relative to available N, such processes would deplete ectomycorrhizal plants in ^{15}N and enrich mycorrhizal fungi in ^{15}N. The central role of glutamine in fungal amino acid metabolism suggests that isotopic fractionations associated with glutamine creation and transformation may control the large ^{15}N depletion between ectomycorrhizal fungi and plants. Glutamine, glutamate, and alanine are commonly invoked as probable transfer compounds of nitrogen between ectomycorrhizal fungi and plants (France and Reid, 1983; Smith and Smith, 1990), whereas ammonia is a suspected transfer compound in arbuscular mycorrhizal fungi (Bago et al., 2001). The amido group of fungally derived glutamine may be the source N assimilated by ectomycorrhizal plants (Smith and Smith, 1990) and is the source for nitrogen in N-acetylglucosamine (Zalkin and Smith, 1998), the monomer of the important fungal carbohydrate chitin. The ^{15}N depletion of chitin appears to be a general phenomenon accompanying chitin biosynthesis,

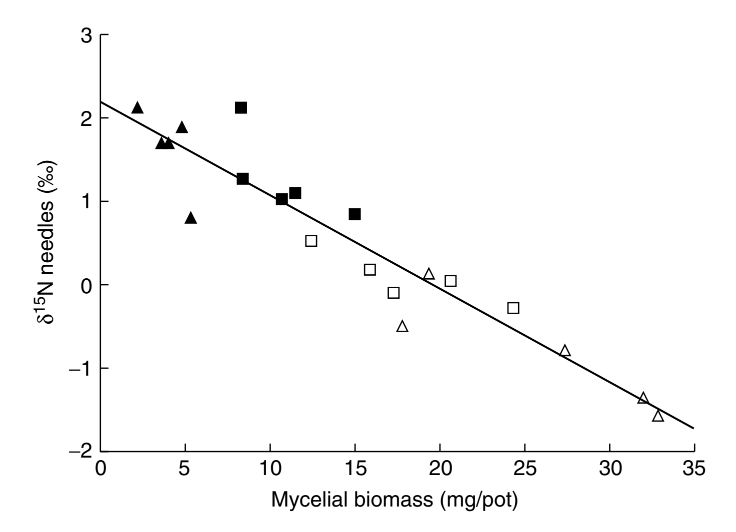


Figure 18.5 Mycelial biomass correlates with foliar ^{15}N in mycorrhizal *Pinus sylvestris*. Fungal biomass in perlite calculated from ergosterol measurements and appropriate conversion factors for *Thelephora* or *Suillus*. $r^2 = 0.90$, $p < 0.001$. High N, filled symbols; low N, empty symbols; triangles, *Suillus*; squares, *Thelephora*. (From Hobbie and Colpaert, *New Phytologist*, 157, 115–126, 2003.)

as chitin in arthropods, marine invertebrates, or fungi is depleted in ^{15}N relative to muscle, total biomass, or protein by 9 to 12‰ (Schimmelman and DeNiro, 1986; Macko et al., 1989; Taylor et al., 1997). It appears probable that kinetic isotopic effects associated with movement of ammonia or amino groups can deplete host plants in ^{15}N relative to fungal symbionts. Such a process would also enrich mycorrhizal fungi in ^{15}N relative to saprotrophic fungi.

The influence of discrimination against ^{15}N during uptake could also influence ^{15}N patterns, particularly if N is supplied in excess of plant and fungal demands. Because culture studies generally apply N at levels higher than in the field, substantial fractionation against ^{15}N on uptake and assimilation is possible (Handley and Raven, 1992; Fogel and Cifuentes, 1993; Emmerton et al., 2001). Therefore, unless nitrogen supply is carefully limited or matched to uptake rates, $\delta^{15}N$ patterns in culture have uncertain relevance to field situations, where N supply rates appear generally low. Fractionation against ^{15}N on uptake could also possibly affect fungal $\delta^{15}N$ in the field if nitrogen no longer limits fungal growth. For example, such fractionation was estimated at 9‰ from dramatic declines in algal $\delta^{15}N$ following nutrient addition in an Arctic stream (Peterson et al., 1993), and similar processes may partially account for wide variations in $\delta^{15}N$ among many taxa of ectomycorrhizal fungi subject to anthropogenic nitrogen deposition in Alaska (Lilleskov et al., 2002). An additional confounding factor is that conditions in the field that encourage fractionation against ^{15}N during uptake (such as higher mineral N concentrations) also favor losses of ^{15}N-depleted nitrogen through ammonia volatilization, nitrate leaching, or denitrification, thereby increasing the $\delta^{15}N$ of the remaining system N.

The effect of ectomycorrhizal colonization on plant $\delta^{15}N$ was recently inferred for three dipterocarp species grown on homogeneous mineral soil, with increasing colonization of root tips associated with declining plant $\delta^{15}N$ (Figure 18.6). The slope in this study suggested that full mycorrhizal colonization would decrease foliar $\delta^{15}N$ by about 7 to 8‰ relative to uncolonized plants.

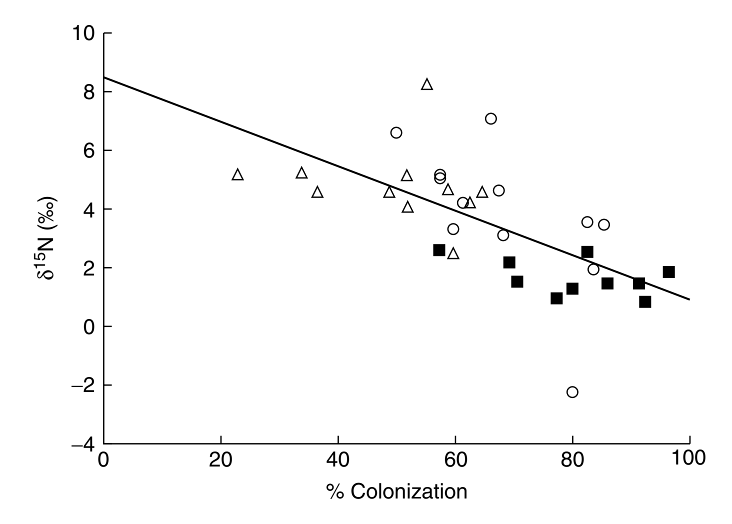


Figure 18.6 Percent ectomycorrhizal colonization of fine roots in three Dipterocarpaceae species correlates with foliar ^{15}N ($r^2 = 0.41$, $n = 33$, $p < 0.001$, $^{15}N_{foliage} = -7.6 \pm 1.6‰ \times$ %colonization + $8.5 \pm 1.1‰$). Species were grown on mineral soil with a ^{15}N of 8.5 ± 1.1‰. Species indicated by *Parashorea tomentella*, open circles; *Hopea nervosa*, open triangles; and *Dryobalanops lanceolata*, filled squares. (Redrawn from Brearley et al., *New Phytologist*, 160, 101–110, 2003.)

The potential for mycorrhizae to influence plant δ^{15}N in the field is clearly shown in Figure 18.7, a study of plant succession after glacial retreat (E. Hobbie and A. Jumpponen, unpublished data). Nonmycorrhizal plants and plants generally colonized by ectomycorrhizal, ericoid, or arbuscular fungi showed similar δ^{15}N values very early in succession, corresponding to low colonization levels of all plant species (Trowbridge and Jumpponen, 2004). Subsequent colonization of plants by ectomycorrhizal and ericoid fungi correlated with a 5 to 6‰ decline in ^{15}N content, again indicating transfer of ^{15}N-depleted N from fungi to plants.

In this study, most ectomycorrhizal and saprotrophic fungi had similar ^{15}N content, about 3 to 4‰ enriched in ^{15}N relative to uncolonized plants. Given that both mycorrhizal and saprotrophic fungi were 3 to 4‰ enriched in ^{15}N relative to putatively nonmycorrhizal plants, this level of enrichment cannot necessarily be attributed to fungal transfer of ^{15}N-depleted compounds to plants, but may instead reflect a general enrichment in ^{15}N of fungal fruiting bodies relative to that of assimilated N. In reference to the last point, Kohzu et al. (2000) reported in a culture study that mycorrhizal fruiting bodies (*Suillus granulatus*) were 2.7‰ enriched relative to mycelia, whereas Handley et al. (1996) reported that caps, but not stipes, of a saprotrophic *Agrocybe* sp. were 1‰ enriched in ^{15}N relative to hyphae (rhizomorphs). No other data directly comparing hyphae and fruiting bodies are available.

A few of the ectomycorrhizal fungi, notably the *Cortinarius*, were dramatically enriched in ^{15}N. Mycorrhizal fungi with high δ^{15}N values often possess proteolytic capabilities (Lilleskov et al., 2002). One possible explanation of this pattern is that mycorrhizal fungi with proteolytic capabilities should assimilate a variety of amino acids, and that ^{15}N from these amino acids is fractionated against during processing to the amino acid forms that are actually transferred to plants, whereas such isotopic fractionation does not occur in fungi directly assimilating ammonium into glutamate and glutamine. However, even in culture studies where mineral nitrogen is the only available nitrogen source, pines colonized by *Suillus* or *Thelephora* were still depleted in ^{15}N relative to supplied nitrogen,

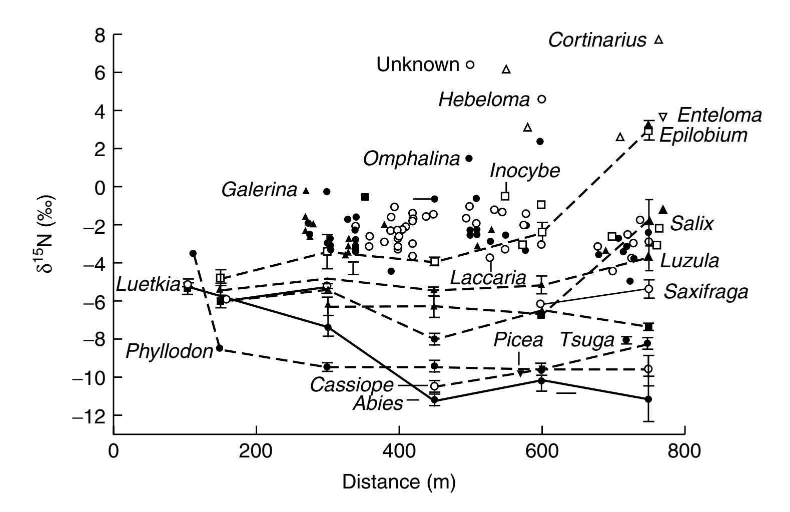


Figure 18.7 ^{15}N content reflects development of mycorrhizae and nitrogen dynamics during primary succession. ^{15}N patterns in plants and fungi after glacial retreat at Lyman Glacier, WA. (Data from E. Hobbie and A. Jumpponen, unpublished.) x-axis indicates distance from current glacial terminus; site ages range from 20 to 100 years (Jumpponen et al., 1998). Mycorrhizal types for plants are ericoid, *Cassiope*, and *Phyllodon*; ectomycorrhizal, *Abies*, *Picea*, and *Salix*; arbuscular, *Epilobium* and *Luetkia*; nonmycorrhizal, *Luzula* and *Saxifraga*. *Cortinarius*, *Entoloma*, *Hebeloma*, *Inocybe*, and *Laccaria* are ectomycorrhizal fungi, whereas *Galerina* and *Omphalina* are saprotrophic fungi. Values for plants are ± standard error (n = 5 typically); values for *Picea* and fungi represent single specimens.

whereas nonmycorrhizal pines had similar ^{15}N content to supplied nitrogen (Hobbie and Colpaert, 2003). Clearly, our understanding of how N isotope patterns are created in mycorrhizal systems requires further mechanistic study. Natural abundance culture studies of mycorrhizal plants grown on protein as sole N sources would be particularly informative, although restricting the confounding influence of bacterial growth in such systems will be difficult (J. Colpaert, personal communication).

Many aspects of $\delta^{15}N$ patterns in ecosystems are still unclear, including (1) whether soil-derived mineral N, free amino acids, and protein differ in ^{15}N content; (2) how relative N availability and fungal N metabolism may influence fractionation against ^{15}N during synthesis of transfer compounds by mycorrhizal fungi; and (3) whether sporocarp $\delta^{15}N$ reflects the overall $\delta^{15}N$ of the vegetative mycelia. Resolution of these issues will require culture studies of saprotrophic fungi, mycorrhizal fungi, and mycorrhizal plants on different N sources of known ^{15}N content, and will also require more compound-specific measures of ^{15}N content in terrestrial ecosystems than done to date.

18.2.4 Mycoheterotrophic Plants

Mycoheterotrophic plants have lost much of the ability to photosynthesize and instead rely primarily on nutrients and carbon obtained from mycorrhizal fungi. Because these plants are yet to be successfully cultured, little is known with certainty about how nutrients and carbon are transferred from fungi to plants. Several recent reports indicate that mycoheterotrophic plants in the Orchidaceae and Monotropaceae possess unusual isotopic compositions, with high ^{13}C and ^{15}N content relative to associated autotrophic plants and putative fungal hosts (Gebauer and Meyer, 2003; Trudell et al., 2003; Figure 18.8). Incidental reports in earlier studies also indicated high $\delta^{15}N$ in mycoheterotrophic plants

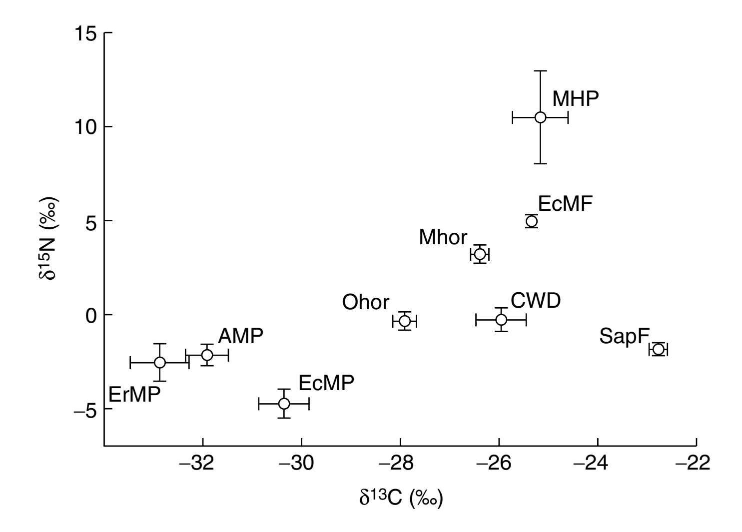


Figure 18.8 Nitrogen and carbon stable isotope values for nine ecosystem pools from two areas in Olympic National Park, WA, including mycoheterotrophic plants. (From Trudell et al., *New Phytologist*, 160, 391–401, 2003, with permission from Blackwell Publishing.) Error bars represent 95% confidence intervals. Abbreviations: AMP, arbuscular mycorrhizal plants; CWD, coarse woody debris; EcMF, ectomycorrhizal fungi; EcMP, ectomycorrhizal plants; ErMP, ericoid mycorrhizal plants; Mhor, upper mineral soil; MHP, mycoheterotrophic plants; Ohor, soil O-horizon; SapF, saprotrophic fungi.

relative to associated autotrophic plants (Delwiche et al., 1979; Virginia and Delwiche, 1982). Ziegler (1995) also reported high $\delta^{13}C$ and δD in such plants. In their study on orchids, Gebauer and Meyer (2003) concluded that $\delta^{13}C$ and $\delta^{15}N$ values could be used to estimate the proportion of nitrogen and carbon derived from associated fungi in putatively autotrophic orchids, with the importance of such heterotrophic nutrition highest in the shade-adapted orchid *Cephalanthera damasonium*. Based on a consistent ^{15}N enrichment in protein and amino acids relative to chitin in other studies, Trudell et al. (2003) suggested that the high $\delta^{15}N$ of mycoheterotrophic plants relative to possible fungal symbionts reflected preferential incorporation of protein-derived nitrogen vs. chitin-derived nitrogen. Given current uncertainties about mechanisms of carbon and nitrogen transfer between fungi and mycoheterotrophic plants and the difficulties of culturing these plants (Smith and Read, 1997), isotopic patterns from field-collected specimens are a promising avenue to investigate the types of compounds assimilated by these plants. For example, based on distinct isotopic patterns in plants grown heterotrophically using either stored lipids or stored carbohydrates (Luo and Sternberg, 1994), it should be possible to determine if mycoheterotrophic plants receive their carbon as lipids or as carbohydrates.

18.3 COMPOUND-SPECIFIC MEASUREMENTS AND ISOTOPIC TRACERS

Few fungi other than those producing large fruiting bodies can be directly sampled for bulk analyses of isotopic ratios. In addition, those fungi that do fruit tend to do so only at certain times of year in response to specific environmental cues, such as sufficient soil moisture or adequate carbon allocation from host plants. Mycologists therefore need

techniques that can provide isotopic information about specific taxa or functional groups through *in situ* sampling of soil. Compound-specific measurements that can be linked to broad taxonomic groups are one possibility, including analyses of phospholipid fatty acids (Cifuentes and Salata, 2001) or fungal cell wall components such as N-acetylglucosamine (S. Frey, personal communication). Methods for the isotopic analyses of DNA or RNA have also been developed (Coffin et al., 1990) and could be applied at different levels of taxonomic resolution (MacGregor et al., 2002). Such analyses have been used in studies of bacterial processes in sediments and aquatic systems, but have been used little in soils. RNA could be particularly useful because it degrades rapidly in the environment and reflects the component of microbial biomass that is actively synthesizing proteins. To date, these techniques have not supplied great insight into fungal processes because uncertainties in what controls isotopic fractionation at the biochemical level have hindered the interpretation of isotopic patterns.

Coupling compound-specific measurements to tracer studies can overcome uncertainties in isotopic fractionation and has considerable promise for understanding the fungal impact on a variety of biogeochemical processes, such as degradation of aromatic compounds (Johnsen et al., 2002) or formation of fatty acids in soils (Lichtfouse et al., 1995). Such measurements can also be used to examine relative activities of fungal vs. bacterial communities on ^{13}C-enriched substrates (Arao, 1999). This general approach is reviewed in Jones and Bradford (2001). Nuclear magnetic resonance (NMR) studies also have great potential for new insights into metabolic processes, are nondestructive, and can provide position-specific information on labeling patterns within molecules that is extremely difficult to obtain using isotope ratio mass spectrometry. Such NMR studies have used ^{15}N- or ^{13}C-labeled substrates to label litter or soil (Clinton et al., 1995; Lundberg et al., 2001). In the latter study, ^{13}C-labeled glucose was traced for 28 days into solid-state components (NMR-invisible components of microbial biomass), respired CO_2, and triacylglycerols, with the triacylglycerols probably located in oil droplets within fungi.

18.4 CONCLUSIONS AND FUTURE RESEARCH

This review should stimulate further applications of isotopic techniques to the roles of fungi in ecosystem processes. Several challenges remain. Because research to date has focused primarily on temperate and boreal coniferous ecosystems, it is unclear if insights gained from these systems can be directly applied to research in other ecosystem types. Field studies in other ecosystems are therefore desirable. In such work, mycologists and ecosystem ecologists should plan integrated studies to address how fungi influence ecosystem function. Similar collaborations between laboratory- and field-based mycologists could increase the rigor of field studies and the relevance of laboratory studies.

Our ability to interpret natural abundance isotopic results from culture studies must be improved through better knowledge of the main metabolic pathways of fungi, and improved understanding of fractionation against ^{15}N during uptake under N-limited vs. non-N-limited conditions. In addition, fractionation against ^{15}N and ^{13}C between fruit bodies and mycelia has yet to be studied systematically in cultures. Once these tasks are accomplished, results from culture studies can then be extrapolated to field studies with greater confidence than now possible. Ongoing work, much of it driven by the potential for microbes in industrial production of specific compounds, has demonstrated that the main metabolic fluxes within microbes can be determined by using ^{13}C-labeled substrates and tracking ^{13}C through various metabolites using NMR techniques (Portais and Delort, 2002). Such approaches are also proving fruitful in studies of carbon metabolism in

symbioses between plants and AM fungi (Pfeffer et al., 2001). Similar experiments at natural abundance levels using mass spectrometry could firmly link specific enzymatic reactions to natural abundance patterns in key microbial metabolites such as amino acids or lipids, thereby allowing researchers to move away from the correlative approaches used to date in field studies. Field studies are likely to increasingly rely on compound-specific measurements that can provide some taxonomic resolution, such as analyses of phospholipid fatty acids.

Our ability to interpret isotopic patterns from field studies should also improve through paired natural abundance and tracer studies in cultures. In addition, field studies using ^{15}N tracers to study ecosystem N dynamics would be improved by including ^{15}N measurements of mycorrhizal fungi (e.g., Buchmann et al., 1996; Zeller et al., 2000). And finally, promising efforts to combine isotopic tracers and computer modeling in field studies (e.g., Currie and Nadelhoffer, 1999) could be expanded to computer models of ecosystem function that include key microbial processes (such as PnET-N-DNDC; Li et al., 2000), or expanded to models of soil food web functioning that explicitly include fungi (Moore et al., 1996). Such modeling could be further extended to isotopic predictions at natural abundances (Hobbie et al., 1999b). For example, by using the NESIS model (http://ecosystems.mbl.edu/Research/Models/nesis/welcome.html) developed by Ed Rastetter, isotopic predictions can be created for any model that includes elemental fluxes. Such isotopic predictions could allow researchers to test many hypotheses about the movement of isotopes through soil ecosystems, thereby improving our understanding of fundamental soil processes driven by fungi.

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19

Diversity-Functioning Relationships in Ectomycorrhizal Fungal Communities

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19.1 INTRODUCTION

Since the earliest report by Frank (1885), in which he documented the benefit of ectomycorrhizal fungi for their plant hosts, ecologists have been intrigued by the functional role of these fungi. Ectomycorrhizal fungi are known to play a principal role in supplementing the nutrient and water requirements of their plant hosts. Although these fungi are found on a relatively small number (ca. 3%) of plant taxa (Meyer, 1973), they are disproportionately represented in several plant families (e.g., Pinaceae and Fagaceae) in temperate and boreal forests in the northern hemisphere. The ectomycorrhizal root system is composed of a sheath or mantle of fungal tissue that envelopes the plant root, a network of fungal hyphae within the root (Hartig net), and an extensive ramifying system of hyphae that extends outwardly from the root and into the surrounding soil (extramatrical mycelium). Nutrients are captured by the hyphal network and transferred to the plant host in exchange for carbon (Finlay et al., 1989; Rousseau et al., 1994). In addition to their facilitation of plant nutrient acquisition, ectomycorrhizal fungi are also known to benefit their plant hosts in other ways, including enhanced plant pathogen resistance (Duchesne et al., 1989), increased plant drought tolerance (Parke et al., 1983), and heavy metal protection (Wilkins, 1991; Hartley et al., 1997) (see also, Fomina et al., Chapter 37 and Turneau and Kottke, Chapter 14, this volume). Although the overall importance of ectomycorrhizal fungi for plants is widely recognized, it is only recently that investigations have begun to focus on the functional role of ectomycorrhizal communities.

Most prior research on the functional role of ectomycorrhizal fungi has focused on single species or on what has been described sometimes as a net mycorrhizal effect. However, because multiple species of ectomycorrhizal fungi colonize individual plant host root systems and exert different influences on their hosts, there is likely no single net effect of ectomycorrhizal fungi on plant host performance. Indeed, Douglas fir is estimated to associate with over 2000 species of ectomycorrhizal fungi over its entire range (Trappe, 1977). The shear magnitude of this variation in fungal species on a single plant host portends a wide range of mycorrhizal functional attributes and poses fundamental questions regarding the functional role of ectomycorrhizal diversity from the standpoint of host performance.

In this chapter, we briefly review advances in ectomycorrhizal research that provide some insight into the community-level functioning of these fungi, particularly as it pertains to plant host performance. As a way of understanding the potential functional consequences of changes in ectomycorrhizal community structure, we focus on community-level changes — in particular, diversity — that have occurred in response to pollution. This body of literature exemplifies changes that have occurred in ectomycorrhizal communities over a relatively short time and provides insights into potential functional consequences of such changes. Elaborating on previously published models of ectomycorrhizal community functioning, we propose a conceptual model linking ectomycorrhizal diversity to host plant performance. We also discuss possible future research directions.

19.2 DIVERSITY AND PHYSIOLOGICAL FUNCTIONING OF ECTOMYCORRHIZAL FUNGI

Ectomycorrhizas are formed by an estimated 5000 to 6000 species of fungi worldwide (Molina et al., 1992), as compared with about 150 species of arbuscular mycorrhizal fungi (Schenck and Perez, 1990). Within individual forest stands, where the functional diversity of ectomycorrhizal fungi is little understood, ectomycorrhizal richness is also high. In a review of the literature, Bruns (1995) reported that the diversity of ectomycorrhizal fungi in forest plots averaging 0.1 ha ranged from 13 to 35 species. Although this seems to be a common range for ectomycorrhizal richness at this spatial scale, the numbers can be much higher. As many as 138 ectomycorrhizal fungal species were found in an oak–pine forest in southwestern Virginia (Palmer et al., 1993), and more than 100 ectomycorrhizal morphotypes were described on pine in pine–oak uplands in the New Jersey Pine Barrens (Tuininga, 2000). Likewise, in a single stand of Douglas fir forest, as many as 100 ectomycorrhizal fungal species may co-occur (Trappe and Molina, unpublished results, cited in Allen et al., 1995). This diversity extends down to single lengths of root, where multiple ectomycorrhizal fungal species coexist (Zak and Marx, 1964; Gibson and Deacon, 1988). Such a high degree of ectomycorrhizal fungal diversity suggests that there is potential for significant community-level effects of these fungal associates on host plant performance and potentially on plant community structure and ecosystem functioning.

The remarkably high diversity of ectomycorrhizal fungi corresponds to an equally broad range of physiological functioning by these fungi. A key function of ectomycorrhizal fungi is in their ability to increase resource availability to plants (Smith and Read, 1997). Through an extensive extramatrical mycelium, ectomycorrhizal fungi dramatically increase the root absorptive surface area of plants and enhance plant nitrogen and phosphorus acquisition. From a functional standpoint, ectomycorrhizal fungal species differ in their abilities to take up inorganic nutrients, such as ammonium (Jongbloed et al., 1991) and phosphorus (Dighton et al., 1993). For example, ammonium uptake differed among *Laccaria bicolor*, *Lactarius rufus*, and *Lactarius hepaticus*, as demonstrated by differences

in the kinetic parameters of nutrient uptake (Jongbloed et al., 1991). Similarly, phosphorus uptake rates differed markedly among five ectomycorrhizal species grown in pure culture (Dighton et al., 1993), and uptake of ^{32}P injected into distinct ectomycorrhizal root zones of birch varied according to mycorrhizal species (Dighton and Harrison, 1990). Differences have even been found in the ability of ectomycorrhizal species to access phosphorus from pure mineral substrates (Wallander, 2000). Because a large fraction of nutrients in most temperate forest soils occurs in organic forms that are otherwise unavailable to plants, the ability of ectomycorrhizal fungi to access organic sources of nutrients is of particular interest. Indeed, ectomycorrhizal fungi have been shown to increase mycorrhizal plant biomass and phosphorus concentration (Perez-Moreno and Read, 2000) and increase plant uptake of nitrogen and phosphorus (Tibbett and Sanders, 2002) when grown on naturally occurring organic nutrient sources. Ectomycorrhizal fungi produce a wide array of extracellular enzymes capable of breaking down organic carbon, nitrogen, and phosphorus in litter. Thus, host access to organic nutrient sources depends on their association with a range of ectomycorrhizal fungi that produce extracellular enzymes capable of acquiring organic forms of nutrients. Acquisition of organic forms of nutrients by ectomycorrhizal fungi has been demonstrated via extracellular production of lignases (Bending and Read, 1997), proteinases (Dighton et al., 1987; Abuzinadah and Read, 1989a, 1989b), and phosphatases (Dighton, 1983; Dighton et al., 1987; Antibus et al., 1997). Not only do ectomycorrhizal fungi produce degradative enzymes, but they differ in their abilities to do so (Kroehler et al., 1988; Abuzinadah and Read, 1989a, 1989b; Antibus et al., 1992). For example, Abuzinadah and Read (1989a) found differences in protein utilization among *Hebeloma crustuliniforme*, *Amanita muscaria*, and *Paxillus involutus* on birch (*Betula pendula*) seedlings. Variation in the ability of ectomycorrhizal fungi to express degradative enzymes and use organic nutrient sources suggests that the functional diversity of the ectomycorrhizal community may be important for host nutrition. Indeed, given the heterogeneous nature of differences in the soil organic matter and physiological abilities of ectomycorrhizal species to access these organically bound nutrient stores, host access to organic nutrients may be enhanced by increasing ectomycorrhizal diversity.

19.3 PLANT HOST RESPONSES TO ECTOMYCORRHIZAL DIVERSITY

It is well known that host colonization by ectomycorrhizal fungi can enhance tree growth (Alexander, 1981; Daughtridge et al., 1986; Amaranthus and Perry, 1987; Perry et al., 1990). Moreover, inoculation studies of individual ectomycorrhizal fungi on seedling root systems have shown that different ectomycorrhizal fungi affect seedling growth differently (Danielson and Visser, 1989; Stenström and Ek, 1990; Browning and Whitney, 1991; Burgess et al., 1993). However, there are relatively few studies that have examined individual host plant responses to multiple ectomycorrhizal inoculations. Chu-Chou and Grace (1985) reported that *Pinus radiata* seedlings inoculated with three ectomycorrhizal species had intermediate growth characteristics compared with single species inoculations. A study examining the influence of ectomycorrhizal fungi on competition between conifer species found that the yield of competing host trees increased as the number of ectomycorrhizal fungi increased (Perry et al., 1989). Simultaneous dual inoculations of Douglas fir seedlings found that in one case seedling biomass was higher when two ectomycorrhizal species were present than with one species alone (Parladé and Alvarez, 1993). Likewise, *Pinus patula* seedlings coinoculated with *Laccaria laccata* and *Thelephora terrestris* had higher shoot biomass than plants inoculated with a single fungal species (Reddy and Natarajan,

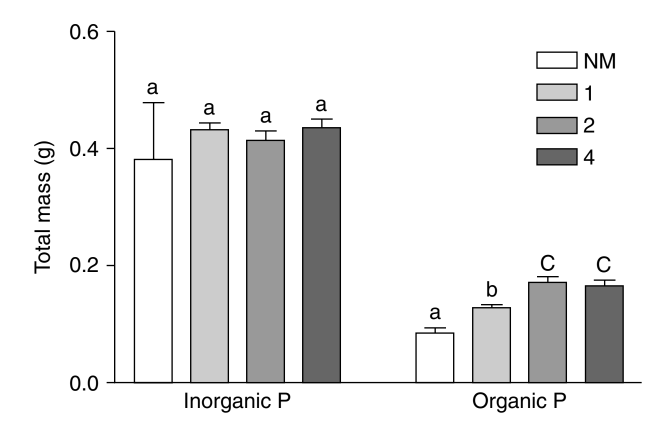


Figure 19.1 Plant biomass (g dry mass) of *P. rigida* grown in either inorganic or organic P conditions colonized with one, two, or four species of ectomycorrhizal fungi or nonmycorrhizal (NM) (mean ± standard error). Significant differences ($p < 0.05$) among diversity levels within phosphorus treatments are indicated by different letters.

1997). While these experiments suggest that the diversity of ectomycorrhizal fungi plays a role in host growth, they do not test host responses to ectomycorrhizal diversity per se.

In one of the first studies examining the effect of mycorrhizal diversity on plants, van der Heijden et al. (1998) reported that plant productivity was at its highest when arbuscular mycorrhizal diversity was highest. Focusing on ectomycorrhizal fungi, Jonsson et al. (2001) reported that birch seedlings (*Betula pendula*) had greater biomass production when inoculated with eight ectomycorrhizal species compared with plants inoculated with single species under low-fertility conditions. Baxter and Dighton (2001) constructed multiple assemblages of one, two, or four species of ectomycorrhizal fungi and showed that ectomycorrhizal diversity per se was a better determinant of improved birch (*Betula populifolia*) seedling nutrient content than species composition or colonization rates. In a subsequent study, Baxter and Dighton (in press) compared pitch pine (*Pinus rigida*) seedlings exposed to an experimental ectomycorrhizal diversity gradient under contrasting inorganic and organic P availability conditions and found that the growth and nutrient uptake response of *P. rigida* to ectomycorrhizal diversity was stronger under organic than inorganic P conditions (Figure 19.1). Despite the positive effect of ectomycorrhizal richness on host performance, there was virtually no effect of shifts in relative abundance of ectomycorrhizal species on host growth or nutrient uptake. These results are intriguing because they suggest that ectomycorrhizal diversity per se, rather than shifts in fungal species composition, increased plant productivity to a greater degree under nutrient-limiting conditions. They also suggest that the effect of diversity was due to greater reliance of the host on ectomycorrhizal access to organically bound phosphorus. Indeed, the six fungal species used in this study differed significantly in surface acid phosphatase activities (Figure 19.2), suggesting that they may differ in their abilities to acquire organic P. Species-specific differences in organic P acquisition by ectomycorrhizal fungi suggest that certain species are better competitors for organic P than others and that host access to organic P may be dependent on the assemblage of fungal species present on the host root system. In a 3-year study examining the effect of ectomycorrhizal diversity on *P. rigida* in unsterilized field soils, Baxter and Dighton (unpublished results) found that, after one growing season, growth and nutrient uptake of *P. rigida* seedlings increased with increasing ectomycorrhizal diversity on tree root systems (Figure 19.3). Again, there was no significant

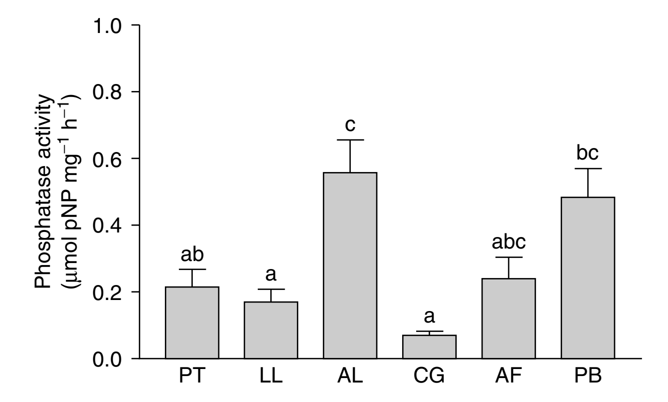


Figure 19.2 Surface acid phosphatase activities (μmol pNP mg^{-1} h^{-1}) of six ectomycorrhizal species on *P. rigida* growing in axenic culture under organic P conditions (mean ± standard error). Fungal species are: PT = *Pisolithus tinctorius*; LL = *Laccaria laccata*; AL = *Amanita flavorubescens*; CG = *Cenococcum geophilum*; AF = *Amanita flavorubescens*; PB = *Piloderma bicolor*. Significant differences ($p < 0.05$) among fungal species are indicated by different letters.

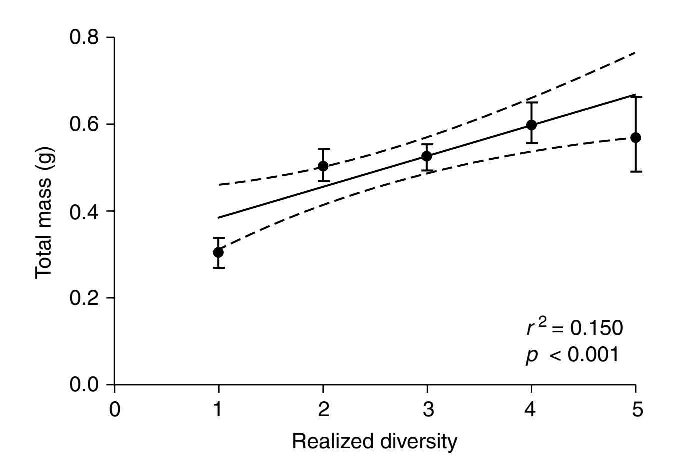


Figure 19.3 Relationship of *P. rigida* total seedling biomass (g dry mass) to realized (at time of harvest) ectomycorrhizal diversity. Seedlings were grown for one growing season (5 mos.) in intact field-collected soil cores (± standard error). Dashed lines are upper and lower 95% confidence limits for the regression.

effect of fungal species composition on host performance. Together, these studies demonstrate that host growth and nutrient uptake respond to ectomycorrhizal diversity but not to shifts in fungal composition. They also indicate that the magnitude of the diversity effect is greater under limiting soil nutrient conditions.

19.4 POLLUTION EFFECTS ON ECTOMYCORRHIZAL DIVERSITY

The dearth of research on the functioning of ectomycorrhizal diversity is particularly important given the increasing impact of pollution and other anthropogenic influences on

temperate forest ecosystems (National Research Council, 1990; Smith, 1990). Numerous studies have shown that human influences on ecosystems can alter the species composition and diversity of fungal communities. Ectomycorrhizal fungi are sensitive to anthropogenic factors, such as pollution and altered nutrient regime. Therefore, human-induced shifts in ectomycorrhizal community structure offer a valuable context for addressing functional changes in these communities. A good example of this is the impact of acidifying pollutants on ectomycorrhizal fungi in forests. Work on the Waldsterben effect of forest dieback in Bavaria suggests that part of the reason for forest tree decline was damage of roots and their ectomycorrhizae by acidifying pollutants (Sobotka, 1964; Ulrich et al., 1979; Hüttermann, 1982, 1985; Blaschke et al., 1985; Stroo and Alexander, 1985). Losses of fungal species with thick sheaths and extensive extramatrical hyphal development appear to be more sensitive to these poullants (Dighton and Skeffington, 1987). Results of this early work on the effects of acid rain on ectomycorrhizae are reviewed in Jansen et al. (1988), Jansen and Dighton (1990), and Dighton and Jansen (1991).

Nitrogen deposition can also alter the abundance, composition, and diversity of ectomycorrhizal fungi. Reductions of both ectomycorrhizal sporocarp production and mycorrhizal root tips have been shown in beech forests in response to forest N fertilization (Rühling and Tyler, 1991; Arnebrant and Söderström, 1992), especially at small differences in N additions to oligotrophic forest ecosystems (Dighton et al., 2004). Species-specific decreases in ectomycorrhizal fungi in Europe have also been observed and attributed to chronic N and acid deposition (Ohtonen et al., 1990; Termorshuizen, 1990; Arnolds, 1991; Brandrud, 1995). Likewise, steep declines have been observed in ectomycorrhizal fungal richness along with changes in fungal species composition in response to N deposition gradients in the northeastern U.S. (Baxter et al., 1999) and Alaska (Lilleskov et al., 2002). Chronic deposition of N to forests may induce limitations or imbalances of other nutrients, such as phosphorus or potassium (Aber et al., 1989; Harrison et al., 1995), which could alter the structure and functioning of ectomycorrhizal communities. If N deposition forces deficiencies of other nutrients, concomitant shifts in ectomycorrhizal community structure may be toward ectomycorrhizal species with superior scavenging abilities for those nutrients in least supply (Lilleskov et al., 2002). Despite substantial evidence of changes in ectomycorrhizal community structure and diversity in response to pollution, we know little about the functional consequences of these changes.

In light of pollution effects and their potential to alter ectomycorrhizal–plant associations, Dighton and Jansen (1991) proposed a conceptual model in which changes in ectomycorrhizal fungal composition or abundance due to pollution alter plant nutrition and carbon balance. Their model is based on the understanding that the mycorrhizal association is affected by both carbohydrate supplies from the host and nutrient levels in the soil. Dighton and Jansen (1991) suggested two mechanisms that could lead to reduced ectomycorrhizal colonization or changes in ectomycorrhizal community structure: (1) a decrease in carbon supply to roots caused by a reduction in photosynthesis in the tree canopy and (2) damage to the root or mycorrhizal fungus that would impair the ability of the absorptive organ to take up nutrients from the soil. In the first mechanism, pollutant-induced reduction in the photosynthetic capacity of the tree canopy reduces the allocation of carbon to roots and their ectomycorrhizae. Reduced energy supply decreases overall mycorrhizal colonization of roots and favors fungal species that can tolerate low carbohydrate supplies. The second mechanism involves damage to the nutrient-absorbing organs themselves. For instance, acidifying pollutants reduce soil pH, which makes toxic metals (e.g., aluminum, manganese, and magnesium) more soluble and, hence, more available to plants (Skeffington and Brown, 1986; Tyler et al., 1987; van Breeman and van Dijk, 1988). This increased toxicity leads to reduced root growth, root dieback, and reduced mycorrhizal

fungal growth and root colonization. Both the reduction in carbohydrate supply and, in particular, changes in soil chemistry can result in altered ectomycorrhizal species composition on tree roots affected by acid rain (Dighton and Skeffington, 1987). If exogenous additions of pollutants or other stresses cause shifts in ectomycorrhizal composition or diversity, with a concomitant change in the functioning of the ectomycorrhizal community, Dighton and Jansen (1991) suggest that plant performance may be altered.

In a related model of stress-induced responses of mycorrhizal host interactions, Anderson and Rygiewicz (1991) proposed that changes in tree physiology occur in response to stress-induced shifts in ectomycorrhizal colonization, but that these changes are compensatory, with the tree reaching a new metabolic homeostasis that maintains the nutritional needs of the plant. The Anderson and Rygiewicz (1991) model suggests that a dynamic homeostasis exists between host and mycorrhizal fungus in terms of source–sink relationships of carbon and that any alteration in plant or fungal physiology caused by an external stressor should change carbon source–sink relationships such that a new metabolic homeostasis is achieved. Hence, the model emphasizes potential feedbacks between the plant and mycorrhizal symbiont, with a compensatory control by the plant host over carbon allocation. Although the model does not directly address the role of mycorrhizal community composition or diversity in relation to carbon allocation, it does highlight the potential for compensatory responses by the host in response to anthropogenic shifts in ectomycorrhizal community structure. Indeed, differences in carbon demand among ectomycorrhizal fungi may be important in determining the degree of fungal vs. host control over carbon allocation.

It is perhaps important in evaluating these models of ectomycorrhizal diversity functioning that on one hand the plant host is an individual, whereas on the other hand the fungal component is a dynamic community, each member of which is genetically and physiologically distinct and exhibits population fluctuations over time. Even within an individual ectomycorrhizal mycelium there exists a high degree of physiological diversity (Cairney and Burke, 1996), with a potential for fungal control over carbon allocation (Wallander, 1995). Indeed, Wallander (1995) suggests that the fungal symbiont has a high degree of control over allocation of carbon to either growth or nitrogen assimilation, depending on soil nutrient availability. The fungal community is also dynamic and can change relatively rapidly in response to changing host or environmental conditions. This suggests that much of the spatial and temporal heterogeneity in soil conditions is integrated in the host by a diverse and dynamic ectomycorrhizal fungal community. By contrast, the host alone is relatively limited physiologically in its ability to adjust to marked changes in soil conditions. In this view, physiological adjustments by the host to altered soil conditions or carbon allocation may be driven by changes in the relative colonization levels of individual species of ectomycorrhizal fungi on the root system.

Because carbon flow in plants is toward the most active sink area (Quick and Schaffer, 1996) and ectomycorrhizal fungi differ in their carbon demands on the host (Cullings et al., 2001; Leake et al., 2001), shifts in ectomycorrhizal diversity should alter carbon sink strength such that carbon is allocated to the most actively growing mycorrhizal sink. Indeed, evidence for differential carbon sink strength among distinct ectomycorrhizal fungi has been observed by Bidartondo et al. (2001). Because multiple ectomycorrhizal fungi colonize individual plant root systems, fungal species do not compete for host carbon in isolation. Once multiple fungi colonize a host, individual species are connected through a common host and must effectively compete with other fungi for host sink strength. The degree to which a dynamic homeostasis (*sensu* Anderson and Rygiewicz, 1991) results, in which a balance in carbon sources and sinks exists within ectomycorrhizal plants, is of particular interest and should be evaluated. Recent evidence supporting carbon transfer

between different host plant species connected belowground by a common ectomycorrhizal fungal network (Simard et al., 1997; Robinson and Fitter, 1999) further challenges our notion of source–sink relations of carbon in plants and their ectomycorrhizal associates. If carbon is transferred between plant species via mycorrhizal connections belowground and driven by interspecific source–sink relationships, then effective competition for sink strength by ectomycorrhizal fungi could also depend on the degree of integration of fungi into a common mycorrhizal network (Robinson and Fitter, 1999). Given our ability to track carbon using isotopic tracers in plants and mycorrhizal fungi, this question should be addressed in future studies.

19.5 A CONCEPTUAL MODEL OF ECTOMYCORRHIZAL DIVERSITY FUNCTIONING

Based on the high degree of ectomycorrhizal fungal diversity on individual plant hosts and differences among these fungi in their physiological functioning, we propose a conceptual model that links ectomycorrhizal diversity to plant host performance through differential physiological functioning (Figure 19.4). Our model is similar to that proposed by Dighton and Jansen (1991), except that we expand the model beyond anthropogenic impacts to include any external environmental factor capable of altering ectomycorrhizal diversity, e.g., drought (Shi et al., 2002) or disturbance (Jones et al., 2003). Furthermore, we propose that the relationship between ectomycorrhizal diversity and host performance is mediated by the differential physiological functioning of individual ectomycorrhizal species. In this sense, our model of ectomycorrhizal diversity-functioning fits into a conceptual framework proposed by Allen et al. (2003), in which the heterogeneity of broadly defined mycorrhizal functional groups (e.g., arbuscular vs. ectomycorrhizal) influences resource exchange between the host and symbiont. Although we focus here on nutrient uptake capacity and carbon demand as key physiological traits that differ among ectomycorrhizal fungi, other key functional traits, such as pathogen resistance, drought tolerance, herbivore defense (Gehring et al., 1997), and heavy metal protection, also vary among fungi. To the extent that these functions provide important benefits to the host, a

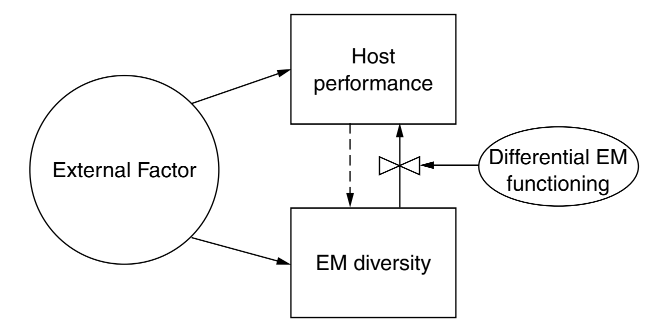


Figure 19.4 Conceptual model of the proposed link between ectomycorrhizal (EM) diversity and plant host performance through differential physiological functioning of individual ectomycorrhizal fungi. External factors (e.g., pollution, drought, or disturbance) may alter host performance directly or indirectly through effects on EM diversity. Likewise, EM diversity may be altered by external factors directly or indirectly through effects on host performance. The dotted arrow indicates the pathway for plant host feedbacks on EM diversity via changes in root carbon allocation.

change in mycorrhizal diversity or composition is tantamount to a change in function. Hence, our model includes any physiological trait that differs among ectomycorrhizal species and affects host performance. We also include the potential for feedbacks from the plant host in the form of root carbon allocation. Feedbacks are driven by shifts in mycorrhizal root carbon sink strength in response to changes in ectomycorrhizal diversity. The model is also based on the observation that ectomycorrhizal fungi differ in their sensitivities to external environmental factors. In our model, external environmental factors may alter host performance directly or indirectly through effects on ectomycorrhizal diversity. Likewise, ectomycorrhizal diversity may be altered by external factors directly or indirectly through effects on host performance.

Given the emphasis in our discussion on the differential access to soil nutrients by ectomycorrhizal fungi, our model explicitly invokes niche complementarity (Tilman, 1994) as a mechanism by which a functionally diverse ectomycorrhizal community acquires host nutrients. The niche complementarity model asserts that in a heterogeneous habitat species possess distinct functional traits that allow them to use different resource pools and thereby contribute positively to ecosystem functioning. Yet, a high degree of overlap in resource use (i.e., niche overlap) among species will dampen the strength of the diversity-functioning relationship (Bond and Chase, 2002). Because soils are spatially heterogeneous mixtures of a wide range of nutrient sources for ectomycorrhizal fungi, the balance between niche complementarity and niche overlap may determine the strength of diversity-functioning relationships in plant–mycorrhizal associations (Baxter and Dighton, 2001; Jonsson et al., 2001). Indeed, the relative role of these two factors in governing diversity-functioning relationships has been a topic of debate (Lawton and Brown, 1994; Hooper and Vitousek, 1997). However, the relative importance of niche complementarity vs. niche overlap in ectomycorrhizal fungal communities has not been investigated. Given the multiple benefits that ectomycorrhizal fungi impart on their plant hosts and the dynamic nature of ectomycorrhizal communities, the mechanism of niche complementarity can be extended to include a more general partitioning of ecological niche space. For example, niche partitioning may occur on a temporal scale, whereby functional changes in ectomycorrhizal diversity or community structure over time correspond to periods of drought, recovery from disturbance, or responses to herbivore infestations. Hence, the role of external factors and the dynamic response of ectomycorrhizal communities to these factors cannot be separated from the community-level functioning of these fungi. From the standpoint of the host, this dynamic and multifunctional fungal community associated with its root system integrates an incredible degree of spatial and temporal heterogeneity in the host's environment.

Based on our diversity-functioning model, there appear to be three possible functional responses of the host to changes in ectomycorrhizal diversity: positive, negative, or neutral. The first potential response is that there is a generally positive relationship between ectomycorrhizal diversity and host performance. This positive response to ectomycorrhizal diversity is consistent with that of several hypotheses that have been proposed to explain the positive relationship between species diversity and ecosystem function. The diversity–stability hypothesis (MacArthur, 1955; Elton. 1958) proposes a direct linear relationship between species diversity and the rate of some ecosystem process, whereas the rivet hypothesis (Ehrlich and Ehrlich, 1981) and the redundant species hypothesis (Walker, 1992) suggest positive, nonlinear relationships with varying degrees of functional redundancy among species (Lawton and Brown, 1994). In this relationship, increases in ectomycorrhizal diversity would result in a net benefit of the mycorrhizal association for the host. Yet, given that there is a lack of empirical evidence for ectomycorrhizal fungi in support of one of these diversity-functioning hypotheses over another, we do not explicitly identify which hypothesis is likely governing this relationship. Benefits to the host of

increased ectomycorrhizal diversity might include enhanced nutrient acquisition, increased drought tolerance, or improved pathogen resistance. An increase in the richness of the ectomycorrhizal community on the host root system corresponds to an increase in the range of physiological functions imparted on the host by the associated fungal species. By the same token, a decrease in ectomycorrhizal diversity would have a net negative effect on host performance. For example, if ectomycorrhizal species that are sensitive to anthropogenic influences provide unique benefits to the host, a reduction or elimination of these species may impair host functioning. While compensatory responses by tolerant ectomycorrhizal species may occur, the net effect of these changes in diversity would be positive. The magnitude of the effect would depend on the functional redundancy among mycorrhizal species and on the nature of the fungal symbiont and its relative costs and benefits to the host.

The second possible relationship of ectomycorrhizal diversity and host performance would be negative. That is, increases in ectomycorrhizal diversity would result in a decrease in host functioning; likewise, lower ectomycorrhizal diversity would result in increased host performance. This type of negative response by the plant to increasing ectomycorrhizal diversity is functionally similar to a parasitic host response (*sensu* Jones and Smith, 2004). Under some conditions such an inverse relationship may exist. For example, at high levels of ectomycorrhizal richness on an individual host root system, interspecific competition among fungal symbionts (say for host carbon) may be intense. Consequently, at high ectomycorrhizal richness, host performance may be impaired, partially resulting in reduced carbon allocation to the roots. A reduction in root carbon allocation would in turn decrease ectomycorrhizal diversity. A decrease in ectomycorrhizal diversity from a level at which the association is parasitic would then result in enhanced host performance. If this inverse relationship between ectomycorrhizal diversity and host performance does occur, it likely does so at relatively high levels of diversity and only under certain abiotic and biotic circumstances.

A third possible outcome is that no net change in functioning of the host will occur following changes in ectomycorrhizal diversity (i.e., neutral). This neutral response is functionally a null hypothesis and is consistent with the "idiosyncratic" hypothesis proposed by Lawton (1994), in which there is no relationship between species diversity and ecosystem function. In this scenario, shifts in ectomycorrhizal species composition or diversity due to environmental factors would have either no effect on the host or result in compensatory responses by the host. Extending the notion of compensatory responses to ectomycorrhizal colonization (*sensu* Anderson and Rygiewicz, 1991), any compensatory response by the host to changes in ectomycorrhizal diversity would be due to the host adjusting physiologically to functional changes in ectomycorrhizal community structure. Alternatively, compensatory changes could also occur in the fungal community itself. For this to occur, a new ectomycorrhizal community would have to be either functionally similar in its relative costs and benefits to the host, such that no net change in host functioning occurred with changes in diversity, or individual species would themselves have to adjust physiologically. For example, soil nutrient imbalances caused by various pollutants could be ameliorated by compensatory shifts in ectomycorrhizal community structure toward fungal species that are most efficient at acquiring limiting nutrients. Although speculative, some evidence for this outcome has been reported (Lilleskov et al., 2002). Consequently, host responses would be the same regardless of ectomycorrhizal diversity. Determining which of these three functional responses best explains the response of the host to changes in ectomycorrhizal diversity or community composition would clearly advance our understanding of the mechanistic nature of the symbiotic relationship between plant host and ectomycorrhizal fungus.

19.6 CONCLUSIONS AND FUTURE DIRECTIONS

Our understanding of the functional consequences of changes (human induced or otherwise) in ectomycorrhizal diversity or community composition remains unclear. The diversity of ectomycorrhizal fungi, together with their differential capacities to access, supply, and utilize host resources, suggests the potential for a key functional role of ectomycorrhizal fungal diversity for host performance. Recent results indicate that ectomycorrhizal fungal richness per se has a greater influence on host performance than community composition or relative abundance (Baxter and Dighton, 2001). However, additional studies with other fungi and hosts are necessary to fully evaluate the relative role of these components of diversity. We are also still relatively ignorant of the comparative physiology of many ectomycorrhizal fungal species and considerably hampered by our inability to culture a number of ectomycorrhizal genera and species to obtain that physiological information. Future efforts should focus on characterizing physiological differences among ectomycorrhizal fungal species and functional groups and the relationship of these differences to mycorrhizal host physiology and performance. Controlled manipulations of ectomycorrhizal richness, composition, and evenness, together with knowledge of species-specific or functional group–specific physiological traits of component fungi, would significantly advance our understanding of the underlying mechanisms of ectomycorrhizal diversity-functioning relationships. Although we have emphasized differences in nutrient uptake capacity and carbon demand by ectomycorrhizal fungi, other physiological traits should also be compared for their influence on host performance. Drought tolerance, pathogen resistance, herbivore defense, and heavy metal protection are all benefits that ectomycorrhizal fungi can potentially impart to their hosts. To extend our understanding of the role of ectomycorrhizal diversity to natural ecosystems, future studies should also incorporate nutrient limitation, soil resource heterogeneity, and soil organisms into the design and experiments conducted under natural field conditions and over longer periods. Through a combination of traditional microcosm experiments in combination with isotopic tracer studies (see Chapter 18), it should be possible to elucidate the complex relationship between the structure of ectomycorrhizal communities and their ecological functioning.

Experiments that test plausible mechanisms of ectomycorrhizal diversity-functioning relationships are also needed if we are to predict host responses to shifts in ectomycorrhizal diversity and community structure. Despite accumulating evidence demonstrating that distinct ectomycorrhizal fungi differ in their capacities to access and supply nutrients to plants, the functional significance of altered ectomycorrhizal community structure for ecosystem functioning is still a relatively unexplored area of research. Linking changes in host performance to shifts in ectomycorrhizal diversity caused by anthropogenic or other environmental influences will require experiments that distinguish direct environmental effects from diversity effects on host performance. Thus, carefully designed and controlled experiments must be conducted by manipulating both of these factors together. Given the high degree of ectomycorrhizal diversity at a variety of organizational levels and potential threats to this diversity by pollution and human disturbance, a greater understanding of the functional role of ectomycorrhizal diversity is needed.

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20

Fungi, Bacteria, and Viruses as Pathogens of the Fungal Community

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20.1 INTRODUCTION

Fungi may be attacked by a diverse array of organisms and agents, including other fungi, bacteria, viruses, prions, and nematodes. This chapter will cover fungal, bacterial, and viral pathogens, as more is known about these systems. A great deal of literature in this area focuses on the use of these organisms for biological control. In approaching the subject of the effect of these pathogens on the fungal community, one cannot ignore the deep literature base, but it is important that it not become the focus of a chapter such as this one. Because this volume is about the fungal community, we will try to introduce systems for which there is at least some information on the effect of the pathogens on fungal populations. For many systems, this type of information is sparse or completely lacking.

The actual importance of these pathogens to the fungal community is poorly understood. In few cases have extensive surveys been done that correlate pathogen populations to host fungus populations in a broad series of natural settings. So, in effect, many of the examples presented in this chapter represent snapshots of such interactions, often drawn from agricultural situations. In many instances, the mycoparasitic (fungal pathogen–fungal host) system actually represents two parts of a three-part hyperparasitic (fungal parasite–fungal pathogen–plant host) system. Thus, the population biology of the fungal hyperparasitic interaction cannot be examined in the absence of the plant host.

The most complete catalogue of viruses and viral pathogens of fungi was compiled by Buck (1986). Although now somewhat dated, this represents an exhaustive list of virus and virus-like elements identified in fungi up to that time. Sadly, only a few of these

viruses have been characterized in significant detail, and the effects of many on their fungal hosts remain obscure.

The vast number of mycoparasitic fungi and the diversity of hosts they attack are reflected in lists compiled by Jeffries and Young (1994). In contrast, there are fewer descriptions of interactions between parasitic bacteria and fungal hosts, especially regarding effects on fungal communities. Much of what is understood today involves indirect effects on fungal communities and falls into a few discrete categories with respect to agricultural relevance. A distinct group is represented by pathogens of cultivated mushrooms, and another major group involves microbial agents with potential for biological control of plant diseases. The degrees of interaction differ between specific examples of parasite and host within each of these categories and can range from simple antagonism, such as by the production of secondary metabolites with antifungal activity, to complex, symbiotic relationships. The latter portion of this chapter will focus on examples of interactions within each category, especially their relevance to fungal communities.

20.2 VIRUSES AS PATHOGENS OF FUNGAL COMMUNITIES

Viruses are common intracellular associates of fungi. Many fungal viruses are pathogenic in their hosts, but a great number are symptomless. The fact that so many fungal viruses — perhaps the majority — are symptomless is part of the reason they are less thoroughly studied than they might otherwise be. Only when viruses have a visible impact on fungi of economic importance do they become the subjects of any detailed investigation. Even when symptoms of abnormal growth are noted in certain fungal isolates, it is rarely apparent that these are the result of virus infection. Demonstrating unequivocally that a particular virus is the cause of an abnormality in a fungal isolate may take months or years, depending on the fungus and virus. In many respects, viruses of fungi are more difficult to work with in the laboratory than viruses of most other organisms. It is technically challenging to infect virus-free fungal isolates by inoculating them with purified virus particle preparations (i.e., fulfill Koch's postulates). In fact, though experiments toward this goal have been performed on many fungal virus systems, such experiments have been successful very few times. For these reasons, fungal viruses remain very much underinvestigated in terms of their real impact on the fungal community. Still, there are several outstanding examples of systems in which the role of viruses in altering fungal communities is well documented. There is no doubt that many other fungi whose biology on the population scale is greatly affected by viruses have yet to be described.

The viruses that probably have the greatest effect on the fungal community overall belong to the virus families *Metaviridae* (Boeke et al., 2000a) and *Pseudoviridae* (Boeke et al., 2000b). Few mycologists will recognize these names — viruses in these families are better known as retrotransposons. Retrotransposons have a great effect on the evolutionary biology of fungi, as they may be induced by stress or by any of several different developmental signals, but they really cannot be considered pathogens in any classical sense. Therefore, we will not expand upon them in the context of this chapter.

20.2.1 Viruses of the Chestnut Blight Fungus, *Cryphonectria parasitica*

Among the most thoroughly studied fungi in terms of their viruses is *Cryphonectria parasitica*, the ascomycete responsible for chestnut blight (for review, see Anagnostakis, 1987). *C. parasitica* was fairly well adapted to plant hosts (*Castanea mollisima* and *Castanea crenata*) in its native Asia, causing only mild disease, but when it invaded North

America in the early part of the 20th century (Anagnostakis, 1987) and then Europe some 35 years later (for review, see Heiniger and Rigling, 1994), it exploded into epidemics. In both cases, the tree populations and chestnut-related economies were quickly altered. The longer-term outcomes have been different in the two settings: in North America, the fungus virtually eliminated the American chestnut (*Castanea dentata*), whereas in Europe, many European chestnut (*Castanea sativa*) trees survived. Reasons for this difference are complex but include the slightly greater blight resistance in *C. sativa* than in *C. dentata*, the lower level of vegetative compatibility diversity (vc), and the early introduction of viruses into the European population. *C. parasitica* isolates infected with viruses now known to be of Asian origin were identified in 1951, just 13 years after the fungus was first found in Europe (Biraghi, 1951). Such isolates were found a short time later in many parts of Italy and France, leading to the conclusion that such viruses had been in the fungal population in Europe since early in the epidemic (Biraghi, 1954; Grente, 1965).

It is thought that one of the reasons for vc systems in fungi is to serve as barriers for virus transmission between virus isolates and that vc diversity, therefore, serves to limit virus spread through fungal populations (Anagnostakis, 1983). This idea has been difficult to test at the population level, but substantial work at both the experimental and theoretical levels has been done recently by Milgroom and colleagues (Cortesi and Milgroom, 1998; Cortesi et al., 2001; Carbone et al., 2004; Milgroom and Cortesi, 2004). Results from these and several other studies indicate that laboratory-based experiments, while valuable, tend to overestimate the overall importance of vegetative compatibility as a barrier to virus transmission at the population level.

20.2.2 Diversity of Viruses in *C. parasitica*

More distinct viruses have been identified in *Cryphonectria* than in any other fungal genus. The family Hypoviridae, the most commonly found viruses in the fungus, contains four distinct species, each with different genome characteristics (Hillman et al., 2000). These probably comprise more than 90% of the viruses identified to date in *C. parasitica*. Other well-studied viruses identified in the fungus include a mitochondrial virus that is a member of the *Mitovirus* genus of the Narnaviridae family (Polashock and Hillman, 1994; Wickner et al., 2000a), and a member of the Reoviridae family (Enebak et al., 1994; Hillman et al., 2004). Several other less well-studied viruses representing at least three other virus families have been identified in the fungus (Hillman, unpublished).

20.2.3 Release of Transgenic, Virus-Containing Isolates of *C. parasitica* into the Fungal Community

The *Cryphonectria*/virus system is unique in that transgenic fungal isolates bearing cDNA copies of complete viral genomes have been made and released into the environment to test whether this is an effective means of disseminating biological control (Choi and Nuss, 1992; Anagnostakis et al., 1998). In effect, the desire here is to alter the structure of the fungal community by deliberately introducing a deleterious gene that is inherited through the sexual cycle and also has the capacity to continually give rise to autonomously replicating viruses. This represents a major difference from naturally infected isolates, in which viruses are generally inherited through conidia but not through sexual spores. Early results from these experiments suggested that the cDNA copies were somewhat stable in the environment, but that virus spread away from the original site of inoculation was minimal (Anagnostakis et al., 1998). In these earlier experiments, a virulent form of the virus was deployed. Other attempts to alter the fungal community using transgenic isolates bearing cDNA copies of a less virulent virus that does not have as great an effect on fungal reproduction are currently in progress (Chen and Nuss, 1999; D.L. Nuss, personal com-

munication). Regardless of the details, Milgroom and coworkers posit that the strategy will not meet the objective of replacing a largely virus-free population with an infected population (Milgroom and Cortesi, 2004). This conclusion is based on a summary of evidence from intentional releases of naturally virus infected isolates, as well as theoretical population considerations: a broad diversity of vc groups have been represented in some intentional releases of pools of virus-infected strains, yet they have not led to wide-scale virus spread in diverse populations, so the advantage of broadening vc diversity through recombination may be minor. Furthermore, DNA representing the integrated viral genome would be predicted to be identified as deleterious to the fungal population and, thus, selected against. Analysis of results from these releases of transgenic strains over the next few years will contribute significantly to this theoretical base.

20.2.4 Impact of Viruses on Fungal Epidemics: Dutch Elm Disease

The Dutch elm disease epidemic is one of the classic cases in which viruses may have affected population dynamics in the fungal community (for review, see Brasier, 2001). For several reasons, this is a complicated story. The first Dutch elm disease epidemic in Europe and North America in the early 20th century was caused by the dimorphic perithecial ascomycete *Ophiostoma ulmi*. In the latter part of the 20th century, a distinct fungal species that is more aggressive and fitter in temperate climates, *Ophiostoma novo-ulmi*, replaced the weaker pathogen with particularly devastating effect to the elm population (Brasier, 1988). There is good evidence that viruses, which have been called disease (d) factors in this system, negatively affected the *O. novo-ulmi* population in Europe, and thus influenced the Dutch elm disease epidemic in that area (Rogers et al., 1986b). On the epidemic front, viruses spread rapidly within the subpopulation that is mainly clonal and composed of a single vc group (Brasier, 1986b). Viruses were initially somewhat correlated with reduced virulence of *O. novo-ulmi* in North America (Pusey and Wilson, 1981, 1982), but this has not been supported through later research. Rather, virus presence more strongly correlates with reduced infection (Webber, 1987). In this regard, Brasier (1986a) noted that the d-phenotype was substantially lost from isolates during the time between inoculation of elm trees with virus-containing fungal isolates and reisolation of the fungus from xylem of the infected trees. Rogers et al. (1986a, 1988) found that these reisolated fungi had lost double-stranded (ds) RNA segments during the infection process.

The early work on this system revealed one of the main problems with assigning causality of viruses with reduced virulence. In both the North American and European populations, some of the fungal isolates that were most reduced in virulence or aggressiveness contained many dsRNA segments (Pusey and Wilson, 1982; Rogers et al., 1986b). Other natural isolates were found that contained fewer dsRNA segments, and lab-generated single conidial isolates from multi-segment-containing isolates that contained a subset of the full complement of dsRNA segments were identified (Rogers et al., 1986b). Such studies led to the early conclusion that multiple independent viruses were present in *O. novo-ulmi*, that many could be present simultaneously in the same mycelium, and that the different viruses had different effects on the morphology and fitness of the fungus. The finding that these viruses were mitochondrial, an unusual property for viruses, added interest to the story (Rogers et al., 1987). More recently, it was found that all of the viruses of *O. novo-ulmi* characterized to date are related to each other, members of the same family of viruses (Cole et al., 1998; Hong et al., 1998, 1999). This is a remarkable diversity of related but autonomous viruses within a single mycelium.

In addition to the complexity of fungal strains and species and of viruses associated with differential aggressiveness of the Dutch elm disease fungus, transmission of these mitochondrial viruses is associated with *de novo* generation of mitochondrial plasmids

(Charter et al., 1993; Abu-Amero et al., 1995). Just as it is with other fungal virus systems, teasing out the role of each of these elements — viral or plasmid — in *Ophiostoma* requires large sample sizes and a combination of field and somewhat time-consuming laboratory experiments. The demonstration in this and other systems that mitochondrial genomes may be altered upon hyphal anastomosis renders these experiments particularly difficult (Montiero-Vitorello et al., 1995; Polashock et al., 1997; Bertrand, 2000), and transfection systems to address the specific roles of mitochondrial viruses in symptom development are currently unavailable.

20.2.5 Horizontal Transmission of Viruses to Other Species

Some of the most important overarching questions about viral pathogens of the fungal community as a whole are the same questions that are asked about viral pathogens of other organisms. How does horizontal transfer occur between unrelated fungi? How often does horizontal transfer occur between fungi and nonfungal hosts? What are the requirements for adaptation to a new host, whether related or unrelated?

Relatively little has been done to address these questions experimentally, but interesting data have been gathered from several fungal virus systems. The question of the evolutionary origins of fungal viruses fits well within the context of evolutionary origins of other eukaryotic viruses. It is clear that many different lineages of viruses have entered the fungal community independently, evolved, and been transferred within the fungal community (Figure 20.1).

The first viruses known to be associated with fungi and other eukaryotes belong to what is now known as the Partitiviridae family (Ghabrial and Hillman, 1999). These are among the most genetically simple of viruses: two segments of dsRNA, each of approximately 2 kb, contain coding regions for only two genes — a coat protein and an RNA-dependent RNA polymerase (replicase) (Romanos et al., 1981; Oh and Hillman, 1995). Partitiviruses may be the most common of all fungal viruses, being found in many genera and species, but they rarely have been associated with any disease or phenotypic alteration in fungi they infect. Symptomless viruses of plants with similar properties have been identified and placed in the same Partitiviridae virus family (Ghabrial et al., 2000). Phylogenetic similarity of the plant and fungal partitiviruses was confirmed by sequence analysis (Ghabrial et al., 2000; Oh and Hillman, 1995). The plant partitiviruses are particularly interesting: they are unique among plant viruses in being transmitted only through seed and not by any of the traditional means used for plant virus transmission, including grafting. There remains speculation that plant partitiviruses might in fact be viruses of fungal endophytes residing within the infected plant. In some cases, searches for endophytes in partitivirus-containing plants have yielded negative results. To investigate whether endophytic fungi commonly carry partitiviruses, a relatively small sample of 30 endophytic fungi representing several genera and species associated with different grass hosts were examined for virus content (Oh, 1995). Interestingly, no viruses were identified in the true endophytes, though two different partitiviruses were identified in the relatively closely related epiphyte *Atkinsonella hypoxylon*. It is likely that partitiviruses will be identified in endophytic fungi as a broader range of fungi are examined, and it will then be of interest to study in more detail the connection between partitiviruses of endophytic fungi and partitiviruses of plants within the same taxonomic groups.

Comparative analysis of the *C. parasitica* virus, now named *Cryphonectria hypovirus*-1/EP713 (CHV-1/EP713), revealed that its closest relative was also a plant virus, barley yellow mosaic virus, which in turn is related to animal-infecting picornaviruses such as poliovirus (Koonin et al., 1991; Figure 20.1). This analysis has been confirmed and extended with other members of the same virus family (Hillman et al., 1994; Smart et al.,

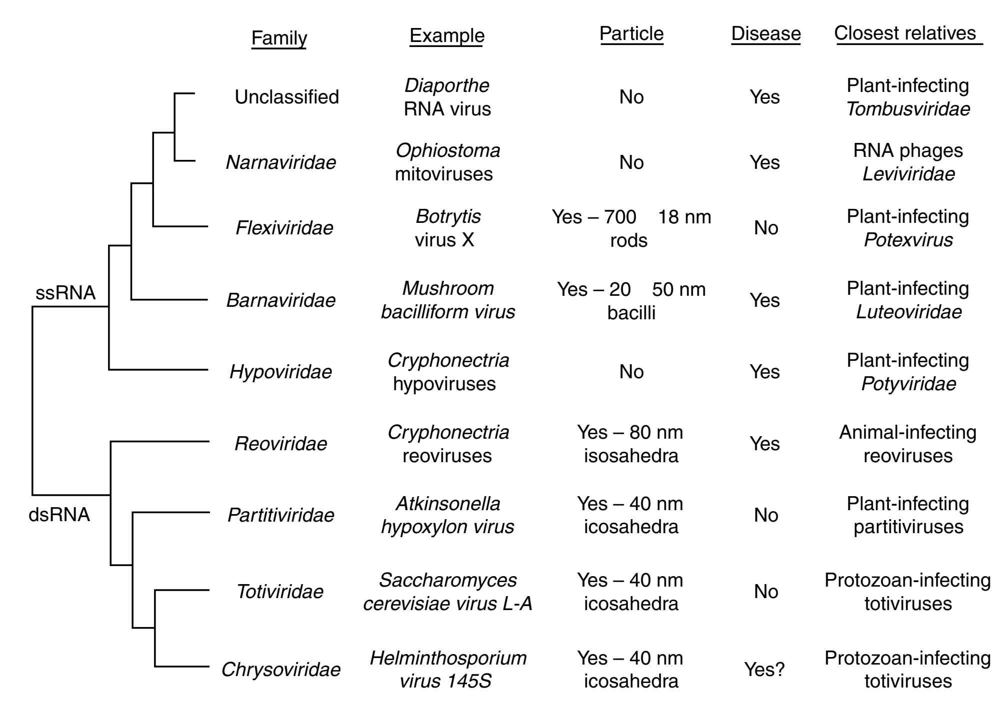


Figure 20.1 Summary of important groups of fungal viruses whose taxonomic status is known. The dendrogram at the left is schematic, based on alignments and dendrogram of RNA-dependent RNA polymerase sequences as presented by Hong et al. (1998) and redrawn by B. Hillman, with addition of sequences for *Botrytis* virus X (Howitt et al., 2001), *Cryphonectria* reoviruses (Hillman et al., 2004; R. Festa and B. Hillman, unpublished), and *Helminthosporium virus 145S* (Ghabrial et al., 2002; also GenBank accession AF297176-AF297179).

1999). Differences in genetic organizations of these fungal, plant, and animal-infecting viruses reflect to some degree the differences in requirements for specific genes in particular host backgrounds. Some of these are obvious, such as the absence of a gene unique to plant viruses that is required for cell-to-cell movement. Other differences are less obvious, for example, the absence of a coat protein gene in the fungal viruses even though other fungal viruses are structurally similar to their plant virus counterparts (Howitt et al., 2001; Figure 20.1).

Now that a number of different fungal virus genomes have been sequenced, it is apparent that lateral transmission from another organism has occurred many times (Figure 20.1). Positive-sense RNA viruses are often subdivided into three supergroups based on RNA polymerase phylogenies (Koonin and Dolja, 1993). Representatives of each of these three polymerase supergroups have been found in the kingdom Fungi (Koonin et al., 1991; Polashock and Hillman, 1994; Howitt et al., 2001). Similarly, representatives of different dsRNA virus families have been identified in fungi and other organisms (Ghabrial et al., 2000; Wickner et al., 2000b; Hillman et al., 2004), supporting the hypothesis of many independent entry events. The closest known relative to a particular fungal virus taxon may be a plant virus (Koonin et al., 1991; Howitt et al., 2001), an animal virus (Hillman et al., 2004), a protozoan virus (Wickner et al., 2000b), or a bacterial virus (Rodriguez-Cousino et al., 1991).

Strong circumstantial evidence for horizontal virus transfer between fungal species comes from recent analysis of a virus from the ascomycete *Sclerotinia homeocarpa* (Deng

et al., 2003). The surprising finding was that the virus was virtually identical to one of the viruses from *O. novo-ulmi*, the Dutch elm disease pathogen. This virus, designated *Ophiostoma mitovirus* 3a (OMV-3a), is one of eight related viruses that have been identified in *O. novo-ulmi* (Buck et al., in press; see above). The OMV-3a isolates from *Sclerotinia* were much more closely related to OMV-3a from *O. novo-ulmi* than the eight *O. novo-ulmi* viruses were to each other. A reasonable hypothesis for the presence of the many related *O. novo-ulmi* viruses is that they evolved from a single progenitor: similar levels of sequence divergence have been observed in the interrelated *C. parasitica* viruses, which very likely evolved from a single progenitor (Smart et al., 1999). The extreme similarity of the OMV-3a sequences from *S. homeocarpa* and *O. novo-ulmi* leads to the inescapable conclusion that presence of the same virus in different taxa was the result of relatively recent lateral transfer of this virus alone, but how this may have occurred is not clear.

The discovery of closely related viruses in sympatric isolates of two different *Cryphonectria* species led Liu et al. (2003) to question whether the viruses diverged from a progenitor at the time of fungal speciation or whether this was the result of lateral transfer between these related fungi. In addition to the fact that these two species were sympatric on chestnut and thus had the opportunity for lateral transmission, two other lines of evidence supported the latter hypothesis. First, the virus phylogenies did not correspond to the fungal phylogenies. That is, two clearly distinct clades were represented by *C. parasitica* and the other *Cryphonectria* species (as yet unnamed), but the viruses from these two species were more closely related to each other. A particular virus from *C. parasitica* may have been more closely related to a virus from *Cryphonectria* sp. than to another *C. parasitica* virus. The other line of evidence for lateral transfer was experimental: virus transmission by anastomosis between the two different fungal species in culture could be demonstrated reproducibly, indicating that such transmission could occur in nature. Experiments such as these using hyphal anastomosis as a vehicle for transmission are inefficient and do not lend themselves to large numbers of fungal isolates. However, the relatively recently demonstrated ability to artificially transfect fungi that are closely or distantly related with viruses or with RNA transcripts representing viral genomes will greatly facilitate such experiments (Chen et al., 1996; Sasaki et al., 2002; Esteban and Fujimura, 2003; Moleleki et al., 2003; Hillman et al., 2004).

20.2.6 Using Viruses as Molecular Markers in the Fungal Community

Rust fungi, which belong to the basidiomycete order Uredinales, are biotrophic fungi that cause disease on many plant hosts. Interestingly, dsRNA viruses appear to be ubiquitous among isolates of many different rust fungi (for review, see Zhang et al. 1994). Virus-like particles were identified in rusts in the early 1970s (Rawlinson and MacLeaan, 1973), and by the mid-1980s, dsRNAs had been identified from several rust species (Newton et al., 1985). A more systematic set of studies to examine rust viruses was initiated by Pryor and colleagues in the late 1980s and early 1990s (Pryor and Boelen, 1987; Dickinson and Pryor, 1989; Dickinson et al., 1990, 1993; Pryor et al., 1990). These studies did not lead to clear definition of specific rust viruses and their effects on individuals because of difficulties resolving mixed infections and performing infectivity assays, but a number of important findings emerged from the investigations. First, in some of the virus-containing species, no isolate that lacked virus was identified. There was no evidence for pathology of any of the viruses on their rust host: of more than 100 dsRNA-containing rust isolates, none showed obvious disease-associated phenotype or reduced fitness. Isogenic virus-free isolates were indistinguishable from their virus-containing counterparts.

Initial studies showing apparent ubiquity and host specificity of viral dsRNAs in some rusts, coupled with the difficulty in differentiating fungal strains based on isozyme

analysis, led to the hypothesis that dsRNA elements might be valuable as cytoplasmic markers for population studies (Dickinson et al., 1990). This was supported by the finding that strain specificity of viruses for a fungal host remained even in fungal strains that infected the same plant host and would presumably have ample opportunity for horizontal transfer of viruses. The rust viruses studied appear to be unusually stable in their hosts; however, results from other fungi cited in this chapter showing relatively common horizontal transmission of viruses suggest that this would be a risky approach that would at best be an adjunct to other methods of fungal strain determination. Little has been published on the rust viruses recently, and the results from rigorous testing of the hypothesis that such cytoplasmic elements could be used as reliable strain markers have not been published.

20.2.7 Fungal Killer Systems

Viruses of the yeast *Saccharomyces cerevisiae* and of the corn smut fungus, *Ustilago maydis*, may confer a so-called killer phenotype to their fungal hosts (Wickner, 2001). In these biologically interesting systems, one segment of a two-segment dsRNA virus encodes replication-associated proteins, while the other segment, which may or may not be present in virus-containing strains, encodes both a secreted protein toxin and a protein conferring resistance to that toxin. The toxins are species specific rather than broad host range: *S. cerevisiae* toxins affect only other strains of *S. cerevisiae*, and *U. maydis* toxins are lethal only to other *U. maydis* strains.

The effect that these killer viruses have on the fungal community as a whole is largely unknown. Killer strains of yeast may cause problems in commercial situations, leading to overall reduction of yeast populations, and many have been found in nature, but specifics of virus ecology are not well studied (Buck, 1986; Kandel, 1988). Much of the early work focused on the killer phenotype itself, which may or may not be virus associated. Similarly in the smut fungi, no thorough ecological survey similar to those done for *Cryphonectria* or *Ophiostoma* viruses has been performed, but smaller surveys have consistently placed the level of killer strains in nature to be around 1% (Buck, 1986; Koltin, 1988). This is well below the high level of natural infection with nonkiller viruses, which are common in *Ustilago* (Koltin, 1988). Killer viruses of yeasts and smut fungi can be thought of in similar terms as fungal endophytes of grasses that are thought to have some beneficial effects on their hosts (White et al., 2002). As a positive selection force, killer viruses likely provide their fungal hosts' selective advantage in niche colonization, though this has not been demonstrated rigorously. As a negative selection force, replication of killer viruses appears to have a slight deleterious effect on their hosts, though there is no overt pathology (Wickner, 2001). Either careful, large-scale studies at the population level or controlled release studies would help to determine the impacts of these competing selection forces.

20.2.8 Other Fungal Virus Systems

A number of important fungal virus systems were not discussed here. For example, viral diseases of cultivated and edible fungi, which may be significant causes of loss to the industry, have been examined in some detail. For a recent review, see Romaine and Goodin (2002). Viruses of the plant pathogenic fungi *Rhizoctonia solani* (Tavantzis et al., 2002), *Helminthosporium victoriae* (Ghabrial et al., 2002), and *Rosellinia necatrix* (Osaki et al., 2002) are among the more important systems for which substantial detail is known. Greater connection between the molecular and population biology of these viruses will continue to add to their interest.

20.3 BACTERIAL AND FUNGAL DISEASES OF FUNGI

20.3.1 Evolution of Interactions between Fungal and Bacterial Parasites of Fungal Hosts

Interfungal and bacterial–fungal relationships are represented by a wide range of interactions that differ in evolved complexity. In both cases, they are strikingly similar to parasitic relationships of plant–microbe interactions (Heath, 1986; Kobayashi and Palumbo, 2000), and thus can be envisioned to follow a similar line of coevolution toward mutualistic symbiosis (Figure 20.2). While there are ample examples of mutualistic symbiosis involving plant–microbe interactions, there are fewer examples involving fungal–microbe interactions. Nonetheless, such examples exist (Pancioni, 1990; Garbaye, 1994). Host–parasite relationships range from simple, less evolved, opportunistic interactions to complex, highly evolved, obligate interactions. These two extremes are separated by examples of relationships that represent facultative parasitic interactions that cannot be considered either simple opportunists or complex obligate parasites.

The simplest of interactions are typically characterized by parasites that display good saprophytic capabilities and a parasitic nature that is opportunistic and destructive to hosts. Examples of simple interactions include bacteria that produce secondary metabolites such as antibiotics or toxins that have an effect on fungi. A slightly more advanced

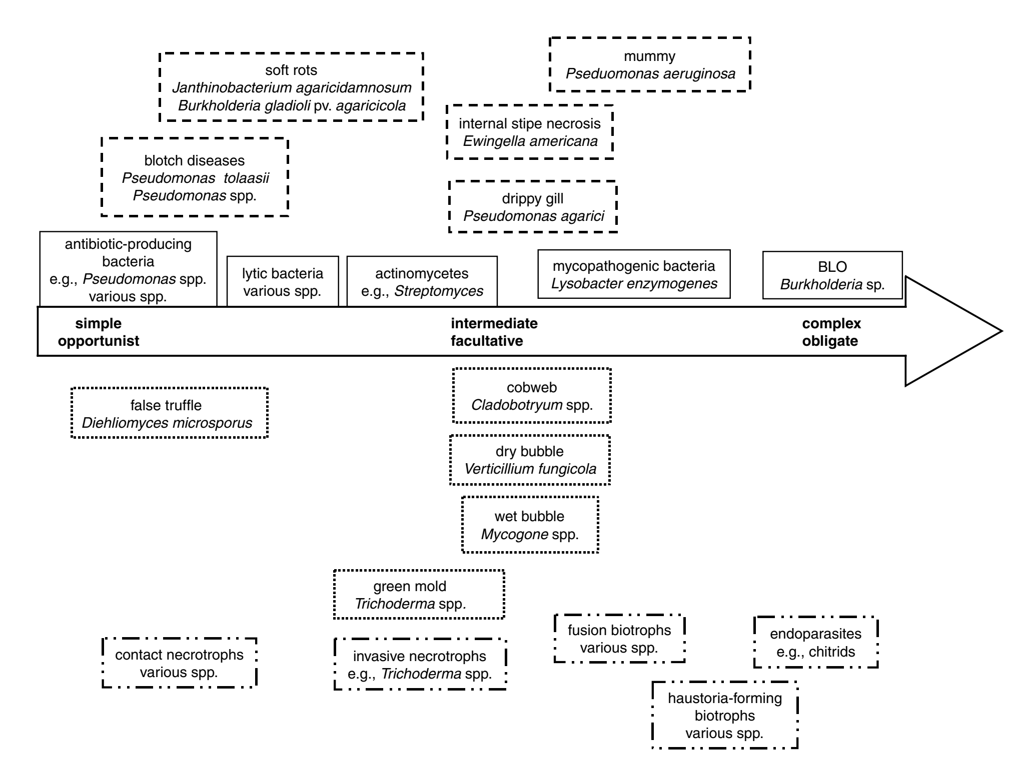


Figure 20.2 Evolution of fungal host–parasite interactions. The central arrow depicts the direction of coevolved complexity toward mutual symbiotic interactions. The locations of boxes representing interaction examples indicate the relative position with line of coevolution. Dashed-line boxes refer to bacterial diseases of cultivated fungi; solid-line boxes refer to bacterial hyperparasites; dotted-line boxes refer to fungal diseases of cultivated fungi; and dashed-dotted-line boxes refer to mycoparasites.

type of interaction is typified by bacteria that produce lytic enzymes directed at fungal cell wall degradation.

The most complex interactions are those involving obligate biotrophs, all of which are positioned on the right side of the spectrum of coevolution depicted in Figure 20.2. Because of their obligate nature, more control of parasitic mechanisms expressed by these parasites is necessary so that less destruction of the host occurs. The specificities required of such interactions typically narrow host ranges for these parasitic types. Among the most advanced interactions of this type appear to be the obligate biotrophs that reside intracellularly in their hosts. These include the endoparasites of the *Chytridiales* (Jeffries and Young, 1994), as well as the obligate bacterial-like organisms (BLOs) observed in mycorrhizal fungi (Bianciotto et al., 1996, 2000). The potential for beneficial activity of BLOs toward their fungal host is clearly in line with an example of mutualistic symbionts of fungi.

Between these two extremes are examples of relationships that represent facultative parasitic interactions. These are best described as interactions of intermediate complexity, which are represented by organisms with saprophytic capabilities, but have adapted to colonizing fungal host tissue as a specific niche. In many cases, the parasitic mechanisms are destructive to the host with seemingly little concern for self-survival. *Trichoderma* spp. and actinomycetes share similar traits for fungal antagonism and represent examples of intermediate or facultative interactions. They are ubiquitous and are aggressive colonizers of soil, reflecting their saprophytic capabilities. However, both *Trichoderma* spp. and actinomycetes clearly display more complex interactions with their hosts and, thus, appear more evolved than simple opportunists. Many mushroom diseases displaying complex interactions also fit into this intermediate interaction category.

Two interesting examples involve the interactions of the bacteria *Lysobacter enzymogenes* and *Pseudomonas aeruginosa* with fungal hosts. These bacteria are set apart from others in that there is strong evidence for true pathogenic interactions with their fungal hosts. While both bacteria are capable of saprophytic survival and express traits consistent with necrotrophy, their pathogenic natures indicate complex interactions requiring intimacy with their fungal hosts, and thus they are positioned as more advanced facultative parasites.

Examples from each of the categories of fungal and bacterial parasites presented in this chapter are represented throughout the spectrum of evolved interactions in Figure 20.2. While one may argue the relative positions of some coevolved interactions, similarities between examples from the different categories are quite evident. There is still much to learn about the various examples, and the recognition of these related interactions will bring better insight into understanding the various interactions.

20.3.2 Bacterial Diseases of Cultivated and Edible Fungi

Bacterial diseases of cultivated mushrooms are caused by a diverse range of bacterial species and may be responsible for substantial loss to the industry. The different symptoms manifested by various diseases reflect the diversity of interactions that occur between pathogens and fungal hosts. While diseases occur on a wide range of fungal hosts, diseases of *Agaricus* are best described in the literature.

Blotch diseases are among the most common bacterial diseases of cultivated mushrooms and are caused by fluorescent pseudomonads that are taxonomically related. Brown blotch, caused by *Pseudomonas tolaasii*, is among the most thoroughly studied in terms of etiology and pathogenesis (Brodey et al., 1991; Rainey et al., 1991; Soler-Rivas et al., 1999). Symptoms of brown blotch typically include a dark brown discoloration that is accompanied by pitted or sunken lesions on mushroom caps and stipes. Symptoms of the related disease ginger blotch, caused by *Pseudomonas gingerii*, include lighter discolor-

ation on mushroom surfaces (Wong et al., 1982). Production of the toxin tolaasin is the primary factor involved in symptom production of brown blotch disease (Rainey et al., 1991). As a result, strains capable of toxin production function essentially as causal agents of the disease. In this case, the simplicity of the interaction allows opportunistic bacterial strains to colonize mushrooms, as suggested by observed taxonomic diversity among causal agents and resulting variations in blotch diseases (Wells et al., 1996; Godfrey et al., 2001a, 2001b; Munsch et al., 2002). The diversity among bacteria capable of producing blotch disease symptoms emphasizes the important point that a diversified group can evolve to aggressively colonize fungal structures as niches.

Diseases that likely represent more complex interactions are those in which bacteria are capable of degrading fungal tissue, as well as internally colonizing fungal structures. These include diseases characterized as soft rots, such as those caused by *Burkholderia gladioli* pv. *agaricicola* and *Janthinobacterium agaricidamnosum* (Lincoln et al., 1991, 1999; Atkey et al., 1992), internal stipe necrosis caused by *Ewingella americana* (Inglis and Peberdy, 1996), and drippy gill disease caused by *Pseudomonas agarici* (Gill and Cole, 2000). While the primary mechanisms of pathogenesis for these disease-causing agents are not well established, a chitinase has been characterized from at least one of these pathogens (*E. americana*) and has been implicated in disease induction (Inglis and Peberdy, 1997).

Among bacterial diseases of mushrooms, mummy disease can be considered to represent the most advanced or most evolved type of interaction. The disease is suggestive of a complex, highly evolved interaction, in that the bacterial pathogen appears to intracellularly colonize the fungal host (Schisler et al., 1968; van Zaayen and Waterreus, 1974). It is not surprising that the causal agent of this disease is reported as *Pseudomonas aeruginosa*, since the pathogenic versatility of the species is evident, regarding both the various organismal hosts it can attack and the variety of mechanisms that are known to contribute to its pathogenic nature (Rahme et al., 1997; Mahajan-Miklos et al., 1999; Tan et al., 1999a, 1999b; Plotnikova et al., 2000; Hogan and Kolter, 2002; Pukatzki et al., 2002).

20.3.3 Fungal Diseases of Cultivated and Edible Fungi

Fungi parallel bacteria as pathogens in that a number of them cause diseases of cultivated and edible mushrooms that appear to vary in complexity of interaction with mushroom hosts (Sinden, 1971). Some diseases appear to represent relatively simplistic interactions between the host and pathogen. For example, various *Trichoderma* spp., which are well-described fungal antagonists (Jeffries and Young, 1994; Harman, 2000), are capable of causing disease on mushrooms. Similar to bacterial pathogens that cause blotch diseases, there is taxonomic diversity among *Trichoderma* spp. that cause green mold disease on mushrooms (Sinden, 1971; Jeffries and Young, 1994; Chen et al., 1999; Ospina-Giraldo et al., 1999). The disease can vary in symptoms, which might be reflective of variations in mechanisms of pathogenesis, including production of secondary metabolites and lytic enzymes known to be produced by *Trichoderma* spp., as well as differences in the specific interaction between the pathogen and host. Nonetheless, the level of interaction for the disease as a whole appears simplistic compared with other fungal diseases of mushrooms. The opportunistic nature of *Trichoderma* spp. as pathogens is reflected in their life cycle; they are common inhabitants of soil, and green mold disease often begins with aggressive colonization of compost prior to colonization of the mushroom (Sinden, 1971; Jeffries and Young, 1994).

False truffle, caused by *Diehliomyces microsporus*, is another example of an opportunistic disease in which the interaction of the pathogen and host is a relatively simple one. The disease occurs as a result of the pathogen outcompeting host mycelia during

substrate colonization, thus preventing mushroom formation. While the pathogen does not attack the mushroom basidiocarp directly, it displays antifungal activity toward the mycelium (Sinden, 1971; Jeffries and Young, 1994).

There are a number of examples of diseases that appear to depict more complex interactions with their respective hosts than those of opportunistic pathogens. Cobweb disease caused by *Cladobotryum* spp. (= *Dactylium*, teleomorph: *Hypomyces*) is characterized by growth of the pathogen mycelium over mushrooms, which can become distorted in shape. Eventually a brown discoloration develops that is followed by rot. Dry bubble disease is caused by *Verticillium fungicola*, which produces lytic enzymes capable of degrading mycelial cell walls (Sinden, 1971; Jeffries and Young, 1994; McKay et al., 1999; Calonje et al., 2000). Symptoms include distortion of mushroom, including stipe swelling, and the formation of spots on the mushroom cap. Infection of younger tissue alters mushroom development and causes undifferentiated tissue growth. Wet bubble disease caused by *Mycogone* spp. causes distortion of the mushroom and the formation of amber droplets that may suggest rot of mushroom tissue.

20.3.4 Bacterial Hyperparasites/Pathogens of Fungi

Among the numerous examples of microbial antagonists of fungi that can be considered parasites or pathogens of fungi, some of the best described are hyperparasites of fungal plant pathogens or biocontrol agents of plant diseases. Biocontrol agents function by a variety of mechanisms (Whipps, 2001); however, agents that express traits involved in direct antagonism toward a fungal host, such as the production of lytic enzymes or secondary metabolites with antibiotic-like activities, are the most relevant to this chapter. While all biocontrol agents may not represent specifically demonstrated examples of pathogens or parasites of fungi, their potential net effect on fungal communities is similar. This point is well emphasized in the form of concerns for nontarget effects of biocontrol agents (Cook et al., 1996; Brimner and Boland, 2003). Along these lines, the disease-suppressing nature of biocontrol agents provides one of the more sensitive methods for measuring the effects of microbes on fungal communities, especially those residing in soils.

Many descriptions of interactions between bacterial parasites of fungi come from soil systems. Indeed, fungal lytic properties of soils have been recognized for several years (Lockwood, 1960; Mitchell and Hurwitz, 1965). These lytic activities are often associated with fungal cell wall-degrading enzymes such as chitinases, glucanases, and proteases produced by a wide range of bacterial antagonists, including *Serratia* and *Enterobacter* spp., *Pseudomonas* spp., *Stenotrophomonas* and *Lysobacter* spp., myxobacteria, and various gram-positive bacteria that include *Bacillus* spp., *Arthrobacter*, and actinomycetes (Jones et al., 1986; Ordentlich et al., 1988; Shapira et al., 1989; Inbar and Chet, 1991; Lim et al., 1991; Fridlender et al., 1993; Mavingui and Heulin, 1994; Chernin et al., 1995, 1997; Dunne et al., 1997; Zhang and Yuen, 2000; Bull et al., 2002; Kobayashi et al., 2002; Sullivan, 2003). In addition to degradative enzymes, some secondary metabolites also possess lytic activity, such as biosurfactants produced by *P. fluorescens* that are capable of lysing zoospores of oomycetes (de Souza et al., 2003).

More advanced parasitic interactions, implied by bacterial attachment or colonization of fungal structures, have been described for several years (Huber and Andersen, 1966; Huber et al., 1966; Tu, 1978, 1979; Yang et al., 1994). In fact, methods using this trait as a primary selection for isolating fungal antagonists from soil systems have proven useful. For example, in recognizing the potential for parasitic relationships, Scher and Baker (1980) used a baiting method similar to those used for fungal agents to isolate potential bacterial biocontrol agents against *Fusarium oxysporum*. Variations of this method have been successfully used to select agents for other biocontrol systems (Scher and Baker,

1980; Nesbitt et al., 1981; Toyota and Kimura, 1993; Kobayashi et al., 1995; Kobayashi and El-Barrad, 1996; Valois et al., 1996).

Similar to the examples provided above, a number of studies have described actinomycetes, such as *Streptomyces* spp., as antagonists of fungal plant pathogens (Crawford et al., 1993; Yuan and Crawford, 1995; Valois et al., 1996; El-Tarabily et al., 1997). Actinomycetes are known as prolific producers of antibiotics and extracellular fungal cell wall-degrading enzymes (Crawford et al., 1993; Valois et al., 1996; Challis and Hopwood, 2003). However, the ability of actinomycetes to colonize both hyphae and spores of fungal hosts (El-Tarabily et al., 1997) is more suggestive of complex relationships with fungal hosts than simple antagonism caused by extracellular factors.

In the above examples, colonization is based mainly on observations of extracellular contact; however, in a few instances, bacterial antagonists also have been observed to intracellularly colonize hyphae of fungal hosts. For example, *Rhizobium* spp. with disease-suppressive activity were observed to colonize mycelia of fungi and oomycetes internally (Tu, 1978, 1979). Observations reported by Willoughby (1983a, 1983b) suggested that internal colonization of the chytrid *Karlingia rosea* by a bacterium identified as *Stenotrophomonas* (formerly *Pseudomonas*) *maltophilia* occurred and this colonization eventually led to the death of the host.

Among proposed bacteria pathogenic to fungi, *Lysobacter enzymogenes* provides a most intriguing system. Members of the genus *Lysobacter* have been characterized as prolific producers of enzymes and secondary metabolites and possess lytic activity toward other microorganisms (Christensen and Cook, 1978; Palumbo et al., 2003; Sullivan et al., 2003). Recently, *L. enzymogenes* strains have been demonstrated to function as effective biocontrol agents (Zhang and Yuen, 1999; Yuen and Zhang, 2001; Yuen et al., 2001; Folman et al., 2003; Sullivan et al., 2003). While both cell wall–degrading enzymes and antibiotic activity produced by the bacterium appear to contribute significantly to fungal antagonism (Zhou et al., 2002; Li et al., 2003; Kobayashi et al., 2005), interactions between *L. enzymogenes* and fungal hosts are clearly more complex than simple antagonism. Indeed, genetic evidence of a true pathogenic relationship between *L. enzymogenes* and fungal hosts has been recently identified. Genes encoding a type III secretion system (TTSS), a central factor for pathogenesis that is common among gram-negative bacteria that attack animals and plants (Hueck, 1998; Cornelis and Van Gijsegem, 2000), were identified in *L. enzymogenes* (Reedy and Kobayashi, 2001, 2003). Mutations within the TTSS of *L. enzymogenes* result in altered interactions between the bacterium and fungal host, *Magnaporthe poae*.

P. aeruginosa has been demonstrated to display biological control of plant diseases (Anjaiah et al., 2003). While biocontrol activity is thought to be primarily due to the production of the various secondary metabolites with antifungal activity, the observed antifungal activity may be the result of a much more complex process. In iteration of interactions with mushroom diseases, the pathogenic relationship of *P. aeruginosa* with fungal hosts is not surprising, due to the pathogenic versatility of the bacterium. Consistent with these observations is that *P. aeruginosa* is pathogenic to yeast (Hogan and Kolter, 2002), further expanding the demonstrated host range of the bacterial pathogen.

Not all parasitic interactions between bacteria and fungal hosts are represented as antagonistic relationships. For example, a *Pseudomonas putida* strain was found to stimulate hyphal growth of *Agaricus bisporus* (Rainey, 1991). This stimulatory effect appeared to require actual contact between the bacterium and fungus and was not due to a diffusible compound, such as antibiotics or other related compounds. Similarly, *Pseudomonas* strains were observed to stimulate mycelial development of mycorrhizal fungi (Barea et al., 1998). In addition to these examples, the most advanced interaction described to date between

bacterial parasites and fungal hosts appears to be one of a proposed mutual beneficial relationship. This example involves the obligate BLOs observed in mycorrhizal fungi (Bianciotto et al., 1996, 2000). 16S rDNA analysis indicated that these BLOs were closely related to *Burkholderia* spp. (Bianciotto et al., 1996, 2000; Perotto and Bonfante, 1997). These organisms have been implicated to be associated with helper bacteria that reside internally or endocytotically within hyphae of mycorrhizae and facilitate establishment of mycorrhizal fungi on their host (Garbaye, 1994; Perotto and Bonfante, 1997).

20.4 FUNGAL HYPERPARASITES OR MYCOPARASITES

There is a substantial body of literature (e.g., see Jeffries and Young, 1994; Whipps, 2001) that describes the various mycoparasitic relationships. Rather than recapitulating these more comprehensive listings, two examples that represent opposite ends of the spectrum of interaction types are presented.

Fungal parasites are categorized into two distinct groups, each of which can be subdivided based on the physiological nature of its interaction (Jefferies and Young, 1994). Necrotrophs, which function by obtaining nutrients from killed host tissue, make up the first group. Many necrotrophs function more as opportunists and are represented by organisms that also display good saprotrophic traits. The mechanisms by which necrotrophs acquire nutrients from their hosts are less controlled, and thus they are more destructive to the host. As a result of these generalities, host ranges of necrotrophs are typically broad, as exemplified by *Trichoderma* spp. and *Pythium* spp. (Deacon, 1976; Whipps, 2001). Necrotrophs are further subdivided into groups that function simply by contact, compared with those that have demonstrated invasive capabilities. *Trichoderma* spp. represent examples of invasive necrotrophs, in which destruction of host tissue is part of the process by which nutrients are acquired from the host. *Trichoderma* spp. are among the most thoroughly studied fungi in terms of antagonism toward fungal hosts (Harman, 2000). The various mechanisms by which *Trichoderma* spp. are antagonistic to fungal hosts as well as their effects on fungal populations have been described. For example, Liu and Baker (1980) conducted a number of experiments using the indirect method of measuring inoculum density based on amount of disease caused by *Rhizoctonia solani.* In essence, their studies demonstrated that populations of *Trichoderma* spp. were inversely proportional to *R. solani* populations in soil: *Trichoderma* populations were high in soils that did not support disease, while low in soils that supported disease. The effects of *Trichoderma* spp. on natural communities have also been recognized through rigorously monitoring rhizosphere colonization of various plant hosts. In these studies, select *Trichoderma* strains were not only capable of colonizing many plant species, but also able to displace the natural microflora (Harman, 2000).

Biotrophs make up the second group of mycoparasites and represent those that derive nutrients from living host tissues. Biotrophs are dependent upon host survival, and thus parasitic mechanisms are more controlled and less destructive to host tissues. Biotrophs establish inherently more complex and evolved interactions with their hosts than do necrotrophs. Their highly specialized nature is reflective of host specificities that generally result in narrower host ranges than necrotrophic parasites. Jeffries and Young (1994) subdivide biotrophs into fusion, haustoria-producing, and intracellular parasites. Fusion and haustoria-producing biotrophs are similar in that contact with the host is necessary, and they establish intricate forms of connections that provide nutrient exchange from the host to the parasite. However, fusion biotrophs differ in that host penetration is not a part of the parasitic process, and thus parasitic interactions would appear to be not as complex.

In contrast, endoparasites are fungi that reside in their hosts intracellularly, at least for part of their life cycle.

Sporidesmium sclerotivorum is an obligate, haustoria-forming biotroph that attacks the sclerotia of *Sclerotinia minor*. The general background of this mycoparasite and the biocontrol system in which it was originally discovered and developed is summarized by Adams (1990). Features of this system represent an intriguing case that has been the subject of model analyses, especially in terms of population dynamics (Gubbins and Gilligan, 1996, 1997; Stolk et al., 1998). Both experimental and modeling studies (Adams et al., 1984; Stolk et al., 1998) indicated that population levels of the mycoparasite are dependent upon the presence of fungal host populations, which is in turn influenced by infection of the plant host. Mycoparasite populations rely on the presence of fungal host populations, which influences rate of sclerotia decay; that is, a higher fungal host population that supports a higher mycoparasite population in turn leads to a higher rate of sclerotia decay. Regardless of the decay rate, fungal host populations were found to decrease in the presence of the mycoparasite in both *in vitro* and field studies (Adams et al., 1984; Adams and Fravel, 1990). These results indicate that obligate biotrophs are capable of influencing host populations in a fashion similar to that of invasive necrotrophs.

20.5 CONCLUSIONS

Pathogens of fungi have a major effect on the structure of the community. In some instances, particularly in artificial or agricultural situations, those effects are reasonably well documented. Mostly, though, the effects are much less well quantified, and we are left with the more obvious examples from fungal hosts of sufficient economic importance to warrant detailed study. More recently developed methods of rapid screening of biological samples for a broad range of microbes and for classifying those microbes should encourage research in this arena, perhaps resulting in an expanded view of these systems.

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21

Fungal Endophytes in Terrestrial Communities and Ecosystems

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21.1 INTRODUCTION

During the past two decades biologists have accumulated a substantial understanding of the pair-wise interactions between endophyte-infected grasses and their herbivores, the evolutionary origins of the endophytes, and the biochemistry and physiology of infection (Clay, 1996; White and Morgan-Jones, 1996; Saikkonen et al., 1998; Craven et al., 2001; Clay and Schardl, 2002). Despite this progress, the higher-level community and ecosystem consequences of grass–endophyte associations are not well resolved. Although fungal endophytes can have strong effects on their host plants, the importance of these plant symbionts in structuring pathogen and herbivore assemblages or guilds of predators and parasitoids is unclear. Endophyte infection of one dominant grass can alter the diversity and abundance of co-occurring plant species (Clay and Holah, 1999), but it is not known how widespread these effects are. Here, we explore how endophyte–grass symbioses might affect the structure and function of terrestrial communities, including mediation of both above- and belowground interactions. We also discuss the potential for endophytes to modify the properties of ecosystems, such as productivity and decomposition. Finally, we examine the role of variation among endophyte genotypes (particularly in chemical profiles) in mediating species interactions at the community level.

21.2 NATURAL HISTORY OF FUNGAL ENDOPHYTE–GRASSES SYMBIOSES

In the 1970s, researchers discovered a new type of association between plants and fungi; endophytic fungi symbiotic with C_3 pasture grasses (subfamily Pooideae) were linked with toxicity to livestock (Bacon et al., 1977). The fungi belong to the ascomycete family, Clavicipitaceae (tribe Balansieae), and their asexual derivatives, including the genera *Atkinsonella*, *Balansia*, *Epichloë, Myriogenospora*, and *Neotyphodium* (the asexual form of *Epichloë*, formerly *Acremonium*) (Clay, 1988, 1990; Schardl, 1996). Since then, biologists have found that these endophyte symbioses are very widespread: approximately 20 to 30% of all grass species are estimated to harbor fungal endophytes (Leuchtmann, 1992). The endophytes can protect their hosts from a variety of biotic and abiotic stresses, particularly from herbivore damage. These benefits arise in part from the production of mycotoxins, specifically several classes of alkaloids. In return, the fungal symbionts acquire nutrients from their host plants.

Many endophyte host species have great economic and ecological importance. *Lolium perenne*, *Lolium multiflorum*, *Festuca pratensis*, and *Festuca rubra* are widely planted worldwide for pasture and turf and have become widely naturalized as well. In the U.S., pasture and rangeland constitute 42% of the total land area (DiTomaso, 2000), and tall fescue (*Festuca arundinacea = Lolium arundinaceum*) alone covers over 15 million ha of the eastern U.S., two thirds of which is endophyte infected (Ball et al., 1993). Many hundreds of native grass species also harbor endophytes (White, 1987; Clay and Leuchtmann, 1989; Clay, 1996; Schulthess and Faeth, 1998; Jones et al., 2000; Vinton et al., 2001).

Infected grasses typically support a single endophyte strain that systemically infects aboveground tissues throughout the life span of the plant (Clay, 1990; Schardl, 1996; but see Tsai et al., 1994; Meijer and Leuchtmann, 1999). The mode of reproduction of the endophyte strain influences the costs, benefits, and ultimately the outcome of symbioses formed with grasses. In general, sexually reproducing strains, which are contagious, are less often mutualistic than asexual, noncontagious strains. For example, sexually reproducing *Epichloë* species (choke disease) effectively sterilize *Dactylis glomerata* (orchard grass) and *Bromus* spp. because fungal stromata form on inflorescences (Groppe et al., 1999; Pfender and Alderman, 1999). However, *Neotyphodium* species, asexual anamorphs derived from sexual *Epichloë*, do not produce stromata (Figure 21.1; reviewed by Hill, 1994; but see Moy et al., 2000). Instead, the mycelium grows into ovules and developing seeds, transmitting infection vertically to the next generation. When endophytes are exclusively vertically transmitted (i.e., not contagious), both theory and empirical evidence reveal that these symbioses function primarily as mutualisms (Bacon et al., 1986; Schardl, 1996; Herre et al., 1999; but see Faeth, 2002).

Endophyte–grass symbioses produce alkaloids that can deter both mammalian and insect herbivores (reviewed by Latch, 1993; Breen, 1994; Clay, 1996). Four classes of alkaloids may be produced by the fungi. The ergot alkaloids comprise peptides of lysergic acid and primarily affect mammals (Dahlman et al., 1991), although they confer some resistance to insects (Bush et al., 1997). Lolitrem (indolediterpenoid) alkaloids, such as ergots, function mainly as mammalian toxins (Dahlman et al., 1991; Bush et al., 1997). Ergot alkaloids and lolitrems have been implicated in reduced weight gain, deficient lactation, increased gestation, lowered fertility, impaired thermoregulation, and gangrene in livestock (Bacon et al., 1977; Bacon, 1995; Cross et al., 1995; Paterson et al., 1995). These effects are reflected in an estimated economic cost of endophyte-infected grasses in the U.S. approaching $1 billion per year (Panaccione et al., 2001).



Figure 21.1 Life cycle of the asexually reproducing *N. coenophialum* in *F. arundinacea*. During the vegetative stage of the plant, fungal hyphae occur in the intercellular spaces of leaf sheath cells. During plant seed production, hyphae grow into and infect developing seeds.

Different endophyte compounds are generally associated with insect resistance, including peramine (pyrrolopyrazine) alkaloids, which are present in the majority of endophyte–grass symbioses, and loline (saturated aminopyrrolizidine) alkaloids, which have been found in concentrations as high as 0.8% of plant dry weight (Siegel et al., 1990; Bacon and Hill, 1996; Wilkinson et al., 2000). Several lines of evidence suggest that like other plant defenses, alkaloid levels can be plastic (or inducible), increasing after plants receive damage, such as grazing or leaf clipping (Bultman and Ganey, 1995; Bazely et al., 1997). While total nitrogen concentration in grass leaves can be enhanced by endophytes, free nitrogen (i.e., that available to herbivores) is often reduced compared with uninfected grass (Lyons et al., 1990). Thus, endophyte infection may lower host plant quality via both toxicity and reduced nutrient availability.

Besides discouraging herbivores, endophytic fungi can benefit infected grasses in several additional ways. Fungal endophytes improve their host's ability to acquire soil nutrients (Lyons et al., 1990; Malinowski et al., 2000), increase tolerance to drought stress (Arachevaleta et al., 1989; West, 1994; Elmi and West, 1995; Hill et al., 1996), and enhance growth (De Battista et al., 1990) and competitive ability in the absence of herbivores (Clay et al., 1993).

21.3 ENDOPHYTES AT THE COMMUNITY LEVEL

21.3.1 Aboveground Herbivore Assemblages

Endophytes of grasses may have considerable impacts on the herbivore assemblages associated with host grasses. Grazing livestock, such as cattle, horses, and sheep, are strongly affected physiologically (see above). Similarly, consumption of infected leaves or seeds impaired thermoregulation and reproduction in laboratory rats and mice (Neal and Schmidt, 1985; Zavos et al., 1988; Godfrey et al., 1994), as well as prairie voles (Durham and Tannenbaum, 1998; Fortier et al., 2000), but had only minor effects on white-footed mice (Tannenbaum et al., 1998; Barger and Tannenbaum, 1998). Some birds species appear to prefer uninfected grass seed (Madej and Clay, 1991), and birds fed endophyte-infected grass seeds had reduced fertility and increased mortality under high-stress conditions (Zavos et al., 1993; Conover and Messmer, 1996).

Although the physiological effects of endophyte–grass symbioses appear strong, far less is known about how endophyte symbioses affect the abundances of vertebrates. Endophyte infection has been implicated in declines of both rabbit (Giuliano et al., 1994) and feral sheep (Bazely et al., 1997) populations. Whether these population-level effects translate into changes in community composition at large scales is less clear. Although capture rates of small mammals were much lower in infected plots than in uninfected plots, endophyte infection of *Festuca arundinacea* did not appear to affect the species richness of small mammals (Coley et al., 1995).

Insects adversely affected by endophytes are taxonomically diverse, including Homoptera (aphids, leaf hoppers, chinch bugs), Lepidoptera (sod webworms, armyworms), Orthoptera (e.g., *Acheta domesticus*), and Coleoptera (flea beetles, flour beetles, billbugs [*Sphenophorus parvulus*], Argentine stem weevil [*Listronotus bonariensis*]) (Funk et al., 1983; Ahmad et al., 1987; Dahlman et al., 1991; Bacon and Hill, 1996; Clay, 1996; Saikkonen et al., 1998; Brem and Leuchtmann, 2001). Some species exhibit decreased *preference* for infected grasses (e.g., Barker et al., 1983), while others may feed equally on both types with reduced *performance* on infected compared with uninfected plants (e.g., Ahmad et al., 1987).

Many studies have examined the effects of endophytes on individual herbivores, but few investigations have considered entire herbivore assemblages. Those studies on assemblages suggest strong effects of endophytes. For example, fewer turf grass pests were found in plots of infected *L. perenne* (Funk et al., 1983) and infected *F. arundinacea* (Murphy et al., 1993) relative to uninfected plots. However, taxa often respond divergently: while three leafhopper species and one beetle were less abundant in infected than in uninfected plots of *F. arundinacea*, another leafhopper and an issid were more common in infected plots (Kirfman et al., 1986). Similarly, endophyte symbiosis reduced two aphid species, two leafhoppers, a flea beetle, and Staphylinidae beetles, but had minimal effects on mites, predaceous arthropods, earthworms, Japanese beetles, and three other aphid species (Davidson and Potter, 1995).

21.3.2 Pathogen Assemblages

In addition to their strong effects on herbivores, fungal endophytes may also influence pathogen assemblages. In *F. arundinacea*, endophyte infection reduced seedling blight (*Rhizoctonia zeae*; Gwinn and Gavin, 1992) and crown rust (*Puccinia coronata*; Ford and Kirkpatrick, 1989) relative to uninfected plants, but did not affect *Puccinia graminis* (Welty et al., 1993). Also, *Alternaria* leaf spot was significantly more common on uninfected *Panicum agrostoides* than on infected plants (Clay et al., 1989). When insects are deterred by endophytes are vectors of plant pathogens, endophyte-infected grasses may also expe-

rience a lower incidence of disease (West et al., 1990). For example, Mahmood et al. (1993) found significantly reduced levels of barley yellow dwarf virus in endophyte-infected *F. arundinacea* due to the enhanced resistance to aphid vectors. In addition, Schulthess and Faeth (1998) revealed that more than 400 different endophytic fungi associated with native Arizona fescue (*F. arizonica*) were negatively correlated with infection by the dominant species, *Neotyphodium starrii*. Clearly, further work is needed to assess the effects at the community level, but current data predict that endophytes will reduce the diversity and abundance of plant pathogens.

21.3.3 The Rhizosphere Community

Although endophytic fungi-infecting grasses occur primarily within aboveground host tissues, they may have a significant impact on belowground rhizosphere communities. Recent results have demonstrated that plants' interactions with soil biota can affect community succession, diversity, productivity, and conservation (Packer and Clay, 2000; Van der Putten et al., 2001; Klironomos, 2002). Past research, primarily with *F. arundinacea* and *L. perenne*, has documented significant differences between infected and uninfected plants (or plots) with respect to associated mycorrhizal fungi, parasitic nematodes, root-feeding insects, soilborne pathogens, N-fixing bacteria, and overall soil feedback responses.

Mycorrhizal interactions can alter competitive relationships among plants (Newsham et al., 1995; Marler et al., 1999) and affect overall community diversity (van der Heijden et al., 1998). Chu-Chou et al. (1992) and Guo et al. (1992) found that endophyte infection of *F. arundinacea* suppressed mycorrhizal fungi in the soil and roots. More recently, Muller (2003) reported a similar suppression with endophytes of *L. perenne*. An antagonistic interaction is not surprising given that both symbionts compete for the same plant resources, including carbon. In contrast, Eerens et al. (1998) suggested that leachates from roots of endophyte-infected plants may benefit the mycorrhizae of clover. The importance of mycorrhizal interactions provides a strong argument for additional research into how endophytes might affect these interactions.

Another mutualistic symbiosis occurs between N-fixing bacteria and plants. The long-term persistence and productivity of many grassland associations may depend on the maintenance of N-fixing clover species (*Trifolium* spp.) within the grass sward (Wilson, 1978). Several studies with *L. perenne* suggest that endophytes enhance the competitive displacement of clover, thereby reducing *Rhizobium* populations and nitrogen fixation in the soil (Sutherland and Hoglund, 1990; Hoveland et al., 1997; but see Prestidge et al., 1992). Clay and Holah (1999) also found that clover rapidly disappeared in endophyte-infected, but not uninfected, plots of *F. arundinacea*. Endophyte infection could therefore affect long-term nitrogen dynamics.

Negative feedback with soil communities results in the accumulation of host-specific parasites and pathogens that reduce the abundance of host plants, maintaining or enhancing community diversity (Janzen, 1970; Bever, 1994). Endophyte infection may alter these interactions by suppressing or enhancing soilborne plant pests. For example, Elmi et al. (2000) found complete mortality of the root knot nematode (*Meloidogyne marylandi*) in pots of endophyte-infected, but not uninfected, *F. arundinacea*. Similarly, Eerens et al. (1998) found that numbers of *Paratylenchus* nematodes were higher in pots and pastures of endophyte-free *L. perenne*, and most other studies report similar effects on endoparasitic nematodes (West et al., 1988; Kimmons et al., 1990; Gwinn and Bernard, 1993; Bernard et al., 1997). Some soilborne pathogens are also suppressed by endophyte infection (Gwinn and Gavin, 1992; but see Burpee and Bouton, 1993). In addition, root-feeding insects and detritivores can be inhibited by endophyte infection. For example, survival of Japanese

beetle larvae was reduced in pots of highly infected *F. arundinacea* compared with pots with lower infection levels (Oliver et al., 1990). Similarly, numbers of detritivorous oribatid mites (*Galumna* spp.) were greatly reduced in endophyte-infected fescue relative to uninfected fields (Williver, 1996; Bernard et al., 1997). However, other species appear to be unaffected or enhanced by endophytes (e.g., earthworms, sminthurid Collembola; Davidson and Potter, 1995; Bernard et al., 1997).

By affecting soil organisms, endophyte–grass symbioses may have cascading effects through the entire terrestrial community. Direct and indirect interactions between soil organisms, between plants and soil, and between above- and belowground biota may be made more complex by endophyte infection. For example, Matthews and Clay (2001) found that soil collected from beneath 4-year-old infected *F. arundinacea* plots resulted in significantly less growth of infected fescue than soil from uninfected plots. In contrast, growth of uninfected fescue was unaffected by soil origin. While the exact mechanism of this effect is unknown, endophytes occurring aboveground clearly altered the ability of the soil to support subsequent plant growth (see also Pedersen et al., 1988; Guo et al., 1992). Studies that consider entire soil communities will help uncover the net effects of endophytes on the rhizosphere, and research examining common species in the rhizophere can help isolate the mechanisms underlying these effects.

21.3.4 Plant Consumers: Predictions

From these observations, we predict that endophyte infection will reduce the *abundance* of plant consumers as a direct consequence of the toxicity of endophyte-derived alkaloids. We may also expect a reduction in the *diversity* of consumers if coping with alkaloid toxicity limits the number of taxa able to consume infected plants. Species persisting in plots of infected plants are expected to consist primarily of alkaloid-adapted specialists rather than generalists that subsist on a wide variety of hosts. For example, the bird cherry–oat aphid (*Rhopalosiphum padi*) is reduced by some grass genotype–fungal isolate combinations, suggesting it may drop out of communities containing these genotypes. Because the aphid is not affected by other combinations, it may be able to persist as a specialist, despite the presence of the endophyte (Siegel et al., 1990). Similarly, as the amount of endophyte mycelium varies among different plant tissues (Hinton and Bacon, 1985), species that specialize on tissue that is relatively less infected (e.g, phloem) are expected to be less responsive to the presence of endophytes. As consumers that are highly susceptible to alkaloid toxicity decline, specialist consumers may increase, shifting community composition. If such a shift occurs, then over time infected plants would be expected to accumulate numbers of consumers similar to uninfected plants. Finally, if endophytes reduce the diversity or abundance of the nonhost plants that co-occur with infected grasses (see below), the diversity and abundance of consumers may decline as an indirect effect of reduced plant diversity (see Siemann et al., 1998).

21.3.5 Plant Assemblages: Diversity and Succession

Direct experimental evidence from one system suggests that endophyte–grass symbioses can dramatically alter the composition of the plant assemblage. Clay and Holah (1999) found that plant species richness in plots of *Neotyphodium coenophialum*-infected *F. arundinacea* was 60% that of uninfected plots, mainly due to reduction of herbivory on infected plants and subsequent changes in plant competitive dynamics (Clay and Holah, 2001). In infected plots, *F. arundinacea* maintained high dominance over time (~90% of total biomass), while in uninfected plots *F. arundinacea* was gradually replaced by other grasses (Figure 21.2). Other studies suggest similar effects. For example, turf plots of infected *L. perenne* had a lower percentage cover of other plant species than did uninfected plots (Funk

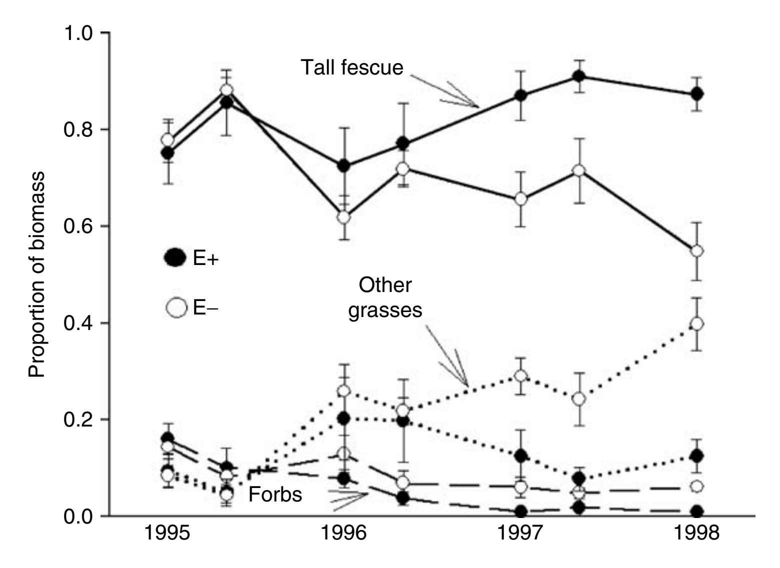


Figure 21.2 Effects of endophyte infection in *F. arundinacea* on the biomass of co-occurring grasses and forbs (Clay and Holah, 1999).

et al., 1983). Similarly, observational data suggested a negative relationship between the infection level of *F. arundinacea* and plant diversity in unmowed field plots; however, a positive relationship appeared in mowed plots (Spyreas et al., 2001). As endophytes were not manipulated in this study, either direction of causation is possible: under mowed conditions, infected *F. arundinacea* may increase plant diversity, or more diverse plots may be more susceptible to invasion by infected *F. arundinacea*. Ultimately, fungal endophytes, particularly those infecting dominant grasses or exotic invaders, may change the trajectory of succession by shifting the composition and diversity of the plant assemblage.

Both direct and indirect mechanisms may underlie the alteration of plant assemblages by endophyte–grass symbioses. First, experiments conducted in the absence of herbivory have shown that endophytes can *directly* enhance the growth and competitive ability of their host (Hill et al., 1991a; Marks et al., 1991; Clay et al., 1993). Differential competitive ability of infected vs. uninfected plants may be especially important under abiotically stressful conditions, as the presence of the endophyte appears to increase the range of ecological conditions under which its host can thrive (i.e., the host's *ecological amplitude*). Second, although experimental evidence is limited, endophytes have been proposed to contribute to the *direct* allelopathic effects of some grass species on other plants, including clover (Peters and Mohammed Zam, 1981; Quigley et al., 1990; Watson et al., 1993; but see Springer, 1996). *Indirect* effects are also likely, as a common consequence of endophyte infection is increased resistance to damage by herbivores and pathogens. These indirect pathways are multifarious and have not been fully disentangled in any system. *Indirect negative effects* are expected if endophytes confer associational susceptibility to co-occurring plants by causing herbivores to switch to more palatable species — this is apparent competition mediated by herbivores. Associational susceptibility appears to occur in plots dominated by infected *F. arundinacea* (Clay and Holah, 2001). However, endophytes–grass symbioses may also function in facilitation, an *indirect positive effect*: when plants occur near infected grasses, they may gain associational resistance (*sensu* Tahvanaian and Root, 1972) via local reductions in the abundance of consumers. That infection frequencies in many species often plateau at around 90% (Shelby and Dalrymple, 1993) suggests that

selection against uninfected plants may be frequency dependent, with uninfected plants gaining a selective advantage via associational resistance at low frequencies.

21.4 MEDIATION OF MULTITROPHIC INTERACTIONS

Endophytes, through their effects on plant enemies such as insect herbivores, may indirectly modify the abundance or composition of higher trophic levels. For example, Japanese beetles were more susceptible to entomopathogenic nematodes when fed on potted infected fescues than when fed on uninfected fescues (Grewal et al., 1995). Alkaloids in artificial diets reduced beetle consumption and mass and enhanced susceptibility to predation (Grewal et al., 1995). Thus, endophytes and predators may act synergistically to reduce consumer populations even more than either factor alone. In a contrasting example, diets of infected grasses reduced the development of Hymenopteran parasitoids of the Argentine stem weevil (*Listronotus bonariensis*) in the laboratory (Barker and Addison, 1996, 1997) and reduced the development (Bultman et al., 1993, 2003) and pupal mass of Hymenopteran parasitoids of *Spodoptera frugiperda* (Bultman et al., 1997), compared with diets of uninfected grass. Similar effects on parasitoids were found when weevils or armyworms were fed artificial diets containing symbiosis-derived alkaloids (Barker and Addison, 1996; Bultman et al., 1997). Thus, by reducing parasitism, endophytes may *indirectly* benefit consumers, potentially counteracting the negative *direct* effects of alkaloids and reduced nutrient availability. Finally, exploring a more complex food web, Omacini et al. (2001) found that infected *Lolium multiflorum* (Italian ryegrass) greatly reduced the rate of parasitism of aphids. Uninfected plants grown outdoors in pots supported more complex food webs and higher parasitoid species richness than infected plants (Omacini et al., 2001).

21.4.1 Potential Mechanisms

A number of different mechanisms could trigger endophyte-mediated changes in terrestrial food web dynamics. First, endophytes may reduce the nutritional quality of the host herbivore (*nutrition hypothesis*). For example, both weevils and armyworms consumed less food when given diets containing endophyte alkaloids (Clay et al., 1985; Barker and Addison, 1996). Second, endophytes may reduce the development time of insect herbivores, making them more susceptible to predation or parasitism when eating infected rather than uninfected grass (*slow growth–high mortality hypothesis*; Grewal et al., 1995; Benrey and Denno, 1997). Endophyte alkaloids may also change other behaviors of herbivores, altering susceptibility to predation (*behavior hypothesis*). For example, queen ants fed infected grass exhibited behaviors characteristic of cattle staggers, a nervous system disorder caused by loline and ergot alkaloids (Tibbets and Faeth, 1999). In contrast, herbivores may sequester mycotoxins from infected plants to use in defense against their natural enemies (*sequestration hypothesis*). Sequestration may occur in the Argentine stem weevil, for which a diet of high alkaloid *L. perenne* reduced the success of parasitoids, compared with uninfected diet (Goldson et al., 2000).

More complex trophic interactions may also occur. For example, the level of herbivore resistance conferred by endophyte infection was reduced in the presence of mycorrhizal fungi in one experiment (Vicari et al., 2002) although the mechanism underlying this pattern is unknown. Endophytes may also alter decomposition rates of host grasses (see below), potentially affecting generalist predators by changing the composition of their prey (e.g., collembola) (Matthews and Clay, 2001; also see Polis and Strong, 1996). Finally, endophytes may increase the accumulation of thatch in areas dominated by infected grasses (Rudgers

and Clay, unpublished data). Increased thatch is known to enhance the abundance of spiders, which can be important predators of herbivores (Gratton and Denno, 2003).

Many questions remain. For example, it is unclear whether the toxic effects of alkaloids typically accumulate or attenuate at higher trophic positions. Considering nonendophyte systems, plant toxins injested by herbivores are sometimes actively sequestered and used in defense against natural enemies (reviewed by Hare, 2002). In contrast, plant secondary compounds can also impair herbivores' abilities to resist their enemies or extend their development, increasing the length of exposure to natural enemies (Hare, 2002). Our ability to predict community changes mediated by endophytes will depend on a deeper understanding of how the feeding guild of herbivores (e.g., leaf chewer, phloem feeder, root parasite) and the level of specialization on endophyte-infected grasses influence the strength of endophytes' effects on higher trophic positions. Finally, we feel that studies in nonagricultural and mixed transmission systems will enhance our understanding of the importance of endophytes to terrestrial food webs (Faeth and Bultman, 2002). Comparisons of endophyte-infected and uninfected grasses in both their native and introduced ranges would be useful in determining the degree to which the benefits (or costs) of infection depend on the coevolutionary history of the grass with its surrounding community. Additionally, in endophyte–grass symbioses with both vertical and horizontal transmission, the benefits of endophytes, as well as the community-level consequences of infection, may be more variable than in systems with exclusively vertical transmission because ecological factors (such as herbivore abundance) can tip the balance between the costs and benefits of hosting an endophyte.

21.4.2 Methods for Examining Food Web Effects

Path analyses based on field surveys of arthropod abundances (see methods in Mitchell, 2001), in addition to field manipulations of organisms at different trophic positions, would aid efforts to determine the relative importance of different mechanisms. Based on the impacts of endophytic fungi on insects, we have generated some testable predictions concerning the food web dynamics sustained on infected vs. uninfected plots of grasses (Figure 21.3). Our predictions are based on work with *F. arundinacea*. First, we expect that interactions between consumers and uninfected grasses will be stronger than those between consumers and infected grasses, as generalist consumers will not be able to cope with infected plants (Figure 21.3). Second, we predict that interactions between consumers and nonhost plant species will be stronger in plots including infected grasses than in plots of uninfected grasses. This pattern would result from generalist consumers switching from eating grass (because it is infected) to eating other plant species. Third, we expect that parasitism and hyperparasitism may be greater on uninfected grasses due to a bottom-up cascade driven by the increased resources available to herbivores on uninfected plants, as Omacini et al. (2001) have proposed. Although the food web diagram presented here is hypothetical, similar diagrams created for specific systems can help tease apart the complex direct and indirect interactions mediated by endophyte–grass symbioses. In addition to path analysis, stable isotope analyses may also prove valuable for examining food web dynamics mediated by endophytes, by determining the diet composition (Sagers et al., 2000) and trophic level (Rundel et al., 1989) of organisms.

21.5 ECOSYSTEM CONSEQUENCES OF FUNGAL ENDOPHYTES

Endophyte infection is clearly capable of having large-scale ecosystem consequences by enhancing the productivity and stress tolerance of host grasses and by altering decompo-

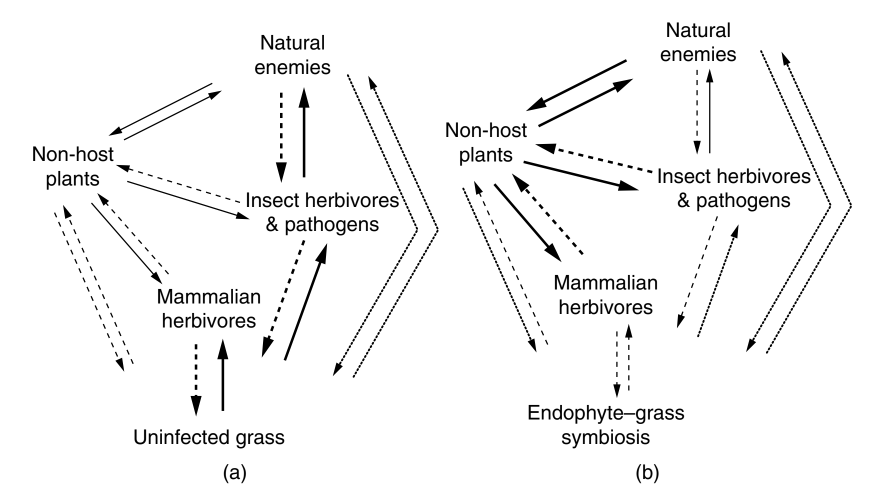


Figure 21.3 Food web diagram of hypothetical interactions between tall fescue and its surrounding community. Solid lines represent a positive effect of one species on another. Dashed lines show a negative effect of one species on another. Dotted lines depict interactions that are currently unresolved (may be positive, neutral, or negative). (a) Interactions in an uninfected tall fescue food web. (b) Interactions in an infected tall fescue food web. Specific differences between the two food webs include (1) a greater consumption of grass relative to co-occurring nonendophytic plants in the uninfected web, compared with the infected web, due to the greater palatability of uninfected grass; and (2) stronger interactions between consumers and their natural enemies on uninfected grasses than on infected grasses due to the greater abundance of consumers on uninfected grasses. Note that the line between the endophyte–grass symbiosis and insect herbivores and pathogens is dotted because the direction of interaction is likely to depend on whether these consumers are specialized to cope with endophyte-produced alkaloids. Note also that in neither web is the indirect interaction between grasses and natural enemies of plant consumers well resolved.

sition and nutrient dynamics. Physiological traits related to water use efficiency and carbon exchange rate are strongly related to growth and biomass production. Thus, endophyte effects on these individual plant traits may have ecosystem-level effects on productivity when endophytes infect grasses that dominate the plant community. In *F. arundinacea*, endophyte infection results in more rapid leaf rolling and greater drought tolerance (Arachevaleta et al., 1989; Bouton et al., 1993; West et al., 1993; but see Barker et al., 1997). Similarly, endophyte-infected *L. perenne* was more persistent and had greater yields at lower rainfall sites in Australia (Cunningham et al., 1993), and infection rate was positively correlated with evapotranspiration rate in France (Grand-Ravel et al., 1995). Several studies have examined photosynthetic rate, measured as carbon exchange rate, in *F. arundinacea*. Belesky et al. (1987) found that photosynthetic rates of uninfected plants were higher than those of infected plants under high light intensity. Richardson et al. (1993) found strong genotypic differences but no overall effect of endophyte infection on photosynthetic rate. Marks and Clay (1996) similarly found strong genotype effects but also reported that infected plants photosynthesized at a significantly higher rate than uninfected plants at high temperatures (35°C), which are not uncommon during the summer within fescue's southeastern range in the U.S. Many studies have documented increased biomass production by infected fescue (for a review, see Clay, 1997). Taken together, these physiological changes can lead to greater persistence and productivity of infected plants relative to uninfected ones.

As discussed above, organisms in the rhizosphere, including N-fixing bacteria and mycorrhizal fungi, can be altered by endophyte infection. Changes in the soil biota may

affect nitrogen dynamics, for example, when N-fixing clover is excluded from infected grass swards (Sutherland and Hoglund, 1990; Clay and Holah, 1999). Although N limitation could ultimately cause the decline of endophyte-dominated grasslands, empirical observations suggest that habitats dominated by infected grasses are recalcitrant to succession compared with uninfected grasslands (Clay and Holah, 1999). As with nitrogen, phosporous dynamics may also depend on endophyte infection of dominant grasses: mycorrhizal fungi are thought primarily to enhance phosphorus uptake (Smith and Read, 1997). When endophytes reduce mycorrhizae (Chu-Chou et al., 1992; Muller, 2003), they may consequently lower phosphorus uptake. In addition to changes in the biotic component of the soil, other, abiotic, effects of endophytes may affect ecosystem properties. Toxic, N-rich alkaloids in endophyte-infected leaf litter could inhibit decomposition (see Bernard et al., 1997) and reduce rates of nutrient cycling. Moreover, in controlled environmental conditions lacking soil microbes, endophyte infection of *F. arundinacea* can directly influence mineral uptake and N use efficiency (Lyons et al., 1990; Malinowski et al., 1997, 2000). These potential changes at the ecosystem level may affect co-occuring plant species as well as their consumers.

Finally, ecological research over the past decade has linked species diversity with ecosystem functioning, advancing our understanding of community dynamics (e.g., Tilman et al., 1997; Chapin et al., 1998; Naeem, 2002). Recent studies have increased the biological realism of prior experiments on diversity by including microbial symbionts of plants (particularly mycorrhizal fungi) in experiments (van der Heijden et al., 1998; Klironomos et al., 2000; Hart et al., 2001). For example, the presence of mycorrhizae can cause increases in primary productivity to level off more quickly with increasing diversity than when mycorrhizae are absent (Klironomos et al., 2000). In a similar way, fungal endophytes, by altering competitive hierarchies and diversity within plant assemblages, may alter the relationship between diversity and ecosystem properties. Importantly, many experiments that have manipulated plant diversity and measured ecosystem responses have been conducted in temperate grasslands, using species in which fungal endophytes are common (Rudgers et al., 2004). However, endophytic fungi have thus far been neglected in studies exploring the effects of diversity on ecosystem functioning.

In both greenhouse and field experiments using *F. arundinacea*, we found that endophytic fungi can alter the relationship between diversity and ecosystem functioning (Rudgers et al., 2004). Specifically, in the field, manipulation of endophyte infection revealed that as plant diversity increased, plots containing uninfected fescue exhibited a greater decline in productivity than did plots containing infected fescue (Figure 21.4). In the greenhouse, manipulations of both endophytes and diversity showed that diversity was negatively correlated with the biomass of uninfected fescue (our measure of fescue's invasiveness) but was not correlated with the biomass of uninfected fescue (Rudgers, Mattingly, and Koslow, unpublished data). Thus, the presence of the endophyte altered the relationship between plant diversity and productivity as well as the relationship between diversity and the community's resistance to invasion by fescue. Given the potential for endophytes to alter the effects of diversity on ecosystem properties, it may be critical to consider these symbionts in experiments and observational studies assessing the importance of species diversity.

21.6 THE ROLE OF FUNGAL GENOTYPE

How endophyte–grass associations structure communities of organisms may depend on fungal genotype. Current evidence suggests that endophyte genotypes differ in compatibility with grass genotypes (Leuchtmann and Clay, 1989; Christensen et al., 1997), production

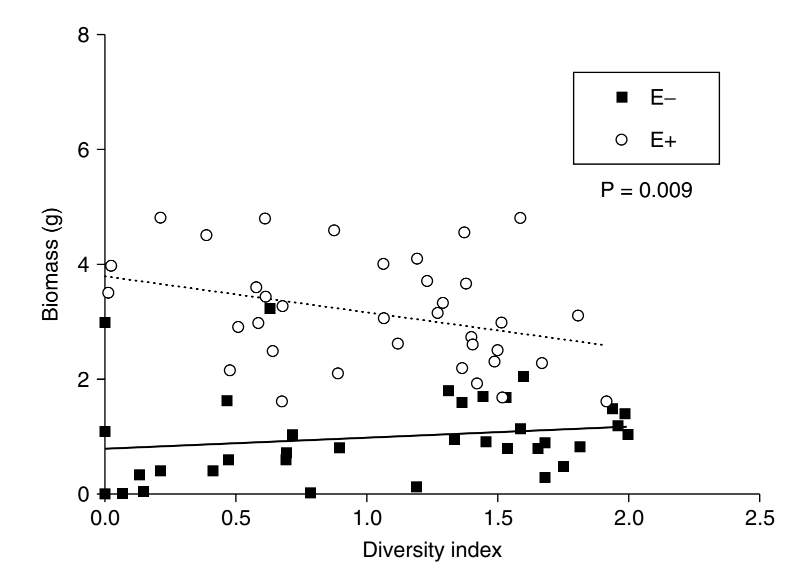


Figure 21.4 Results of a field experiment where tall fescue seeds were added to 20m × 20m field plots that had natural variation in species diversity. Here, we show regression lines (E+ = endophyte-infected, E- = endophyte-free) for residual aboveground total biomass (an estimate of productivity) vs. residual species diversity (Shannon-Weiner diversity index), after other factors in the model have been removed (see details in Rudgers et al., 2004). Each symbol represents a single subplot, and data from two seasons are presented (October 1997 and June 1998; n = 80 subplots/endophyte treatment/date). The significant endophyte × residual species diversity interaction (P = 0.0361) demonstrates that that the endophyte switches the relationship between diversity and productivity. Specifically, the correlation between diversity and productivity was significantly negative in the absence of the endophyte (r = –0.32, P < 0.0001) but nonsignificant in the presence of the endophyte (r = –0.12, P = 0.13). (From Rudgers et al., 2004. With permission.)

of alkaloids (Hill et al., 1991b), resistance to herbivores (Wilkinson et al., 2000; Leuchtmann et al., 2000), production of stroma (Leuchtmann and Clay, 1989; Johnston and Clay, 2003), and their effects on the phenotypic plasticity of grasses (Cheplick, 1998). Fungal genotypes that differ in the types and amounts of alkaloids they produce could have drastically different effects on plant enemies, particularly insect vs. mammalian herbivores. For example, Siegel et al. (1990) found that peramine alone affected one species of aphid, but in other species, combinations of both peramine and loline alkaloids were required for deterrence. Several other experiments also suggest that plant–fungal isolate combinations vary in their effects on aphids (Christensen and Latch, 1991; Clement et al., 1997, 2001). Furthermore, grasses infected with stroma-forming species often have lower alkaloid concentrations than those with asexual endophytes (Leuchtmann et al., 2000), and insect herbivory was greater in tillers that had fungal stromata (horizontal transmission) than in tillers in which endophytes reproduced asexually (vertical transmission) (Brem and Leuchtmann, 2001).

In addition, endophyte alkaloids may affect other members of terrestrial food webs. For example, the percentage parasitism of Argentine stem weevils was inversely correlated with the amount of peramine in *L. perenne* (Goldson et al., 2000). Further, fungal isolates differed in whether they reduced parasitoid emergence from Argentine stem weevils, relative to weevils reared on uninfected grass (Bultman et al., 2003). For some herbivores, diverse, wild-type alkaloid profiles (including ergots) may have additive or synergistic effects: synergism between ergot alkaloids and perloline has been suggested to confer

resistance to milkweed bugs (Yates et al., 1989). Studies elucidating the ecological function of endophyte symbioses with different alkaloid signatures will reveal the importance of fungal genotype at the community level, and ultimately enhance our ability to predict changes in plant, pathogen, herbivore, and predator communities that may accompany the introduction of new fungal genotypes.

Finally, studies that examine the role of fungal genotype will be critical for evaluating advances in the biotechnology of grass–endophyte symbioses. Historically, uninfected varieties of pasture grasses have been planted to moderate the costs of endophyte infection to livestock. Without endophyte mutualists, however, pasture and turf grasses are frequently outcompeted by infected varieties and other plant species (Clay, 1996). A new approach involves introducing endophyte genotypes that lack alkaloids toxic to mammals but that sustain the insect resistance and abiotic stress tolerance conferred by wild-type endophyte genotypes (Ivy et al., 2000). Genetic modification of endophytes to eliminate the production of the mammalian toxin ergovaline has been accomplished (Panaccione et al., 2001), and directly incorporating genes from endophytic fungi into plant genomes has been proposed (Dahlman et al., 1991). To evaluate these advances, we will need to understand how variation in endophyte–grass genotype combinations can differentially affect ecological interactions within and between tropic levels in terrestrial communities.

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22

Mechanisms of Arbuscular Mycorrhizal Mediation of Plant–Plant Interactions

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22.1 INTRODUCTION

Ecologists have historically focused on interspecific competition as the major factor determining plant–plant interactions. While there is abundant evidence that interspecific competition between plants can be strong and important, competition alone does not provide a complete framework for understanding plant community structure (Tilman and Pacala, 1993). Over recent years, a series of studies has suggested that plant–plant interactions could be mediated by interactions with other organisms, including interactions with herbivores (Holt and Lawton, 1994) and pathogens (Van der Putten et al., 1993; Bever, 1994). Many authors have also suggested that plant–plant interactions could be influenced by their interactions with mycorrhizal fungi (see references within this chapter). Mechanisms through which interactions with mycorrhizal fungi could alter plant–plant interactions are the focus of this chapter.

Early work exploring the potential of mycorrhizal fungi to alter plant–plant interactions focused on the possibility that shared mycorrhizal networks could provide conduits for sharing of resources, thereby reducing or altering interspecific interactions (Francis and Read, 1984; Grime et al., 1987). To date, shared networks remain a frequently discussed hypothesis for understanding mycorrhizal mechanisms for the mediation of interactions among plants (see recent reviews by Robinson and Fitter, 1999; Simard et al., 2002). Alternative hypotheses for mycorrhizal mediation of plant interactions have also been suggested, including indirect effects through changes in mycorrhizal fungal density (Janos, 1980) or community composition (Bever, 1999, 2002b), and the possibility that

mycorrhizal fungi facilitate niche differentiation and thereby decrease interspecific competition (Bever et al., 2001; Van der Heijden, 2002; Reynolds et al., 2003).

In this chapter, we review and develop mechanisms for mycorrhizal mediation of plant–plant interactions. In particular, we identify two fundamentally different ways that arbuscular mycorrhizal (AM) fungi can alter plant–plant interactions: (1) through modifying interspecific competition (e.g., resource sharing and mediation of niche differentiation) and (2) through indirect effects mediated by changes in the AM fungal community. As the framework for indirect effects builds on a population ecology perspective of the plant–AM fungal interaction, we first develop this perspective by identifying the separate forces that influence plant and AM fungal population growth rates, and then explore the co-dependence of these factors in a discussion of the range of interactions in individual plant–fungal pairs. We then combine these pairwise modules to infer types of indirect interactions between plant species as mediated through changes in mycorrhizal fungal population densities or community composition.

22.2 BASIC POPULATION ECOLOGY OF THE INTERACTION OF PLANTS AND AM FUNGI

Most plants interact with arbuscular mycorrhizal fungi. This interaction is classically described as mutually beneficial. Plants can benefit from the association through improved uptake of soil nutrients, particularly relatively immobile nutrients such as phosphorus. AM fungi may also improve drought tolerance and resistance to soil pathogens. The fungus, in turn, appears to be solely dependent on the plant for energy. Consequently, the plant–AM fungal interaction is a textbook example of a nutritional mutualism. However, the interaction between plants and AM fungi can range from the fungus parasitizing the plant to the plant parasitizing the fungus. We begin our consideration of these possibilities by first discussing the separate influences on plant and AM fungal population growth rates.

22.2.1 Variation in Plant Response to Mycorrhizal Fungi

Plants are known to vary in their response to mycorrhizal fungi. While some plants and plant families do not associate with mycorrhizal fungi, most associate and depend on arbuscular mycorrhizal fungi, and still others associate and depend on other types of mycorrhizal fungi (e.g., ecto- or ericoid mycorrhizal fungi). There is also variation among plants that interact mutualistically with mycorrhizal fungi. In describing this variation, Janos (1988) has identified a useful distinction between responsiveness of a plant to mycorrhizal fungi (i.e., the net increase in growth with inoculation) and the dependence of a plant (i.e., how much phosphorus fertilizer would have to be added to allow an uncolonized plant to grow similarly to the same plant genotype grown with mycorrhizal fungi). While these two aspects of plant response are likely correlated in that they reflect overall benefit from the association, they are not exactly collinear. Responsiveness of a plant is also a function of many other aspects of plant life history, such as plant growth rate. For example, slow-growing plants may be very dependent on mycorrhizal fungi in that they might have little growth without inoculation. Yet, their growth response may be less than that of a fast-growing plant that is not as dependent on the fungus for its phosphorus acquisition, but able to rapidly translate the incremental increase in phosphorus availability into growth. This is an essential difficulty in measures of plant response and of experiments testing the role of mycorrhizal fungi in mediating plant–plant interactions, particularly when comparing plants of different life histories (e.g., comparison of fast-growing early successional species and slow-growing late successional species). In much

of the current chapter, we will be discussing plant response in the context of the effect of the mycorrhizal fungi on the growth rates of plant populations. In most experimental work, the population consequences are inferred from measures of relative growth rates of individual plants (i.e., measures of responsiveness *sensu* Janos). This is likely a valid approach if plants have similar life histories.

The association between plants and AM fungi is often noted for having low specificity because a given fungus is likely to be able to associate with a broad range of hosts. However, plant response can depend on the species of mycorrhizal fungi with which they are associated. While some fungi are generally more efficient at growth promotion than others, plant growth promotion also depends on the particular pairing of plant and fungus. It has been shown repeatedly that the fungal species that delivers the most benefit to one host may not be the most effective fungus for a second host (Adjoud et al., 1996; Van der Heijden et al., 1998b; Bever, 2002b; Helgason et al., 2002). Therefore, plants can have high specificity of response to AM fungi while having low specificity of association with AM fungi (Bever, 1999; Bever et al., 2002).

Plant response to mycorrhizal fungi also depends critically on the environment. As expected for a nutritional mutualism, plants benefit the most when the nutrient that is being provided is in short supply. As a result, when phosphorus is abundant relative to other plant resources, many plants do not show positive responses to mycorrhizal fungi, and plants may be negatively affected by fungal colonization (i.e., the fungi are parasitic). The environmental dependence of the interaction is interesting, but this will not be the focus of this paper. Rather, for much of the discussion, the abiotic environment is assumed to be constant as we focus on the population consequences of the plant–AM fungal interaction.

22.2.2 Variation in Fungal Response to Plants

All AM fungi are dependent on plants for their growth, with different plant species varying in their overall quality as hosts. In general, there is likely to be a correlation between the overall responsiveness of a plant to AM fungi and their quality as host plants. We found support for such a correlation in comparisons of plant species in an old field, where *Allium vineale* was the most overall responsive plant to AM fungi and was the best host, while *Anthoxanthum odoratum* was generally nonresponsive and was the worst host for AM fungi (Bever, 2002a). We also found that ecotypes of big bluestem from Kansas were more responsive to AM fungi, and also better hosts for AM fungi, than ecotypes from Illinois (Schultz et al., 2001). However, this association of responsiveness and quality of host does not always hold, as illustrated by the observation that mycoheterotrophic plants, plants that derive carbon from the fungus (Leake, 1994), respond positively to mycorrhizal fungi but likely have negative effects on fungal growth rates.

The population growth rates of AM fungi also depend on particular combinations of plants and fungi (again a specificity of response). Evidence for this comes from measures of sporulation on different host plants (Sanders and Fitter, 1992; Bever et al., 1996; Eom et al., 2000). While the importance of spores in fungal life histories may vary among species, host-specific differences in sporulation have been found to represent host-specific differences in fungal population growth rates (Bever, 2002a, 2002b). We observed, for example, that *Scutellospora calospora* had the highest rate of population growth in association with *Plantago lanceolata*, while *Archeospora trappei* had the highest rate of population growth with a second plant species, *Panicum sphaerocarpon*. This observation of specificity of fungal response to plants could reflect preferential association between plant and fungi, as observed by Helgason et al. (2002). Alternatively, the association could be nonspecific and differences in fungal population growth rates could result from differences in benefit the fungus derives per active infection. In the former case, the effect could

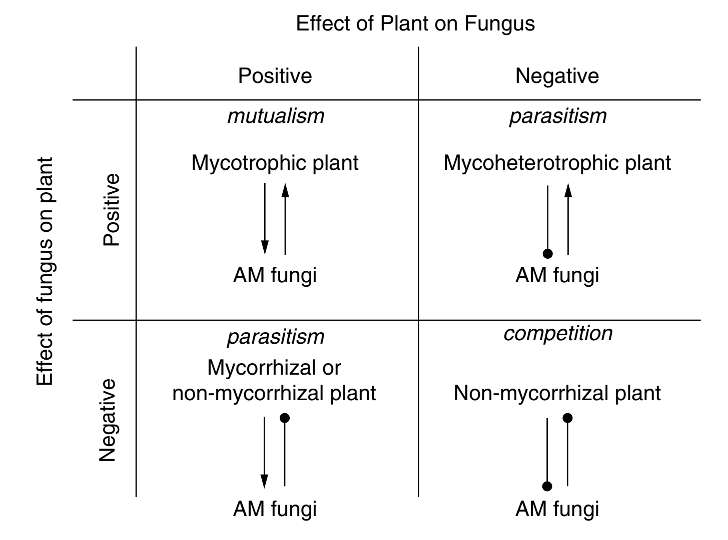


Figure 22.1 Varieties of interactions between plants and AM fungi. The interactions between plants and AM fungi range from mutually beneficial to different forms of antagonism. Under some environmental conditions, facultative mycotrophic plants are negatively affected by AM fungi. Alternatively, mycoheterotrohic plants specialize in parasitizing AM fungi. The interaction between nonmycotrophic and nonmycorrhizal plants and AM fungi could range from pathogenic to potentially competitive.

be mediated by competition between the fungi for infection sites, while in the latter case the effect could be mediated by allocation patterns of the host. Both competition and preferential plant allocation could generate context dependence in the specificity of fungal response to plants, in that the relative growth rates of particular fungi would depend on what other fungi are in the root system. We found evidence of such context dependence when the removal of the dominant fungus, *Acaulospora colossica*, from the root system of *Allium vineale* apparently permitted *Scutellospora calospora*, a previously subordinate fungus, to thrive (Bever, 2002a).

Growth rates of AM fungal populations also depend on the environment. The environmental dependence of AM fungal population growth rates can be due to direct effects of the physical environment on the fungus (e.g., heavy metal toxicity). Alternatively, the environmental influence could be mediated through changes in the physiology of the host plant (e.g., in the case of elevated atmospheric CO_2). Fertilization by phosphorus could cause both direct and indirect effects on fungal population growth rates.

22.2.3 Pairwise Interactions between Plants and AM Fungi

While determinants of population growth of plants and AM fungi need to be considered independently, the discussion above also identifies that plant and AM fungal population growth rates are interdependent, because they are linked by dependencies on each other's densities. These interdependencies can result in a full range of ecological interactions. We briefly consider each of four possible dynamic modules as presented in Figure 22.1.

22.2.3.1 Plant–AM Fungal Mutualism

Many, perhaps most, plant species interact mutualistically with mycorrhizal fungi; i.e., both plant and fungal populations have higher growth rates in the presence of each other.

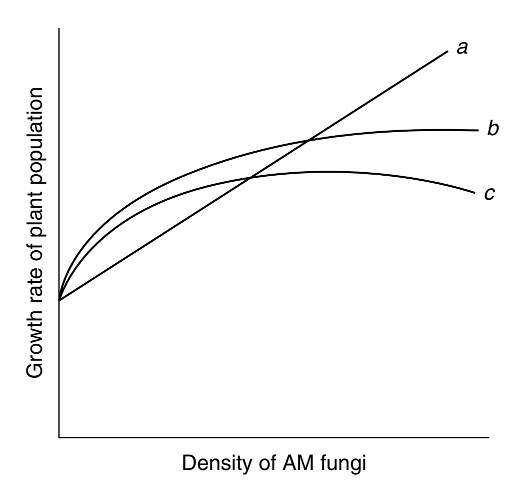


Figure 22.2 Dependence of plant population growth rates on AM fungal density. The dependence of population growth rate on the density of AM fungi could range from linear (line a) to saturating (line b) to having an intermediate maximum (line c).

The benefit that plants receive from the fungus is density dependent, with plants deriving more benefit from the presence of AM fungi at low plant densities than at high plant densities (Koide and Dickie, 2002). Theoretical studies have shown that such density dependence in delivery of benefit can stabilize the dynamics of mutualists (Dean, 1983). It is also likely that the benefit that plants receive from AM fungi depends nonlinearly on the density of AM fungi (Gange and Ayres, 1999). By AM fungal density, we mean measures of AM fungal density in the environment rather than in particular root systems, though colonization in roots is certainly a major component of overall fungal density. While volumes of experimental work have established plant growth promotion due to the presence of AM fungi, less work has investigated the incremental change in plant growth with incremental increases in density of AM fungi. The benefit that plants receive from AM fungi likely saturates at higher AM fungal densities, and it may decrease as the density of AM fungi gets too high (Gange and Ayres, 1999). We represent three potential forms of this relationship in Figure 22.2. This relationship between plant growth and AM fungal density can be a critical issue in consideration of AM fungal mediation of plant–plant interactions through changes of AM fungal density, as discussed below. To date, no one has fit general models of population growth incorporating such density dependencies to the dynamics of particular plant–AM fungal mutualisms.

22.2.3.2 AM Fungal Parasitism of Plants

AM fungi can have negative impacts on mycorrhizal plants, particularly in conditions of high nutrient availability (Johnson et al., 1997; Smith and Read, 1997). While population growth rates of AM fungi may decline with increasing soil resource levels, AM fungi still benefit from association with plants under these conditions. Therefore, the interaction is characterized by fungal parasitism of the plant (Table 22.1). AM fungi can also have negative effects on the growth of weakly mycorrhizal and nonmycorrhizal plants under a broader range of environments. These interactions could also fall into parasitism if the fungi derive some benefit from the plants (see competition discussion below). In parasitic situations, the negative impact of AM fungi likely increases with increasing fungal density, but this has not been tested to our knowledge.

Table 22.1 Expected Direction and Relative Magnitude of Indirect Effects of One Plant Type on a Second Plant through Changes in Density of Mycorrhizal Fungi

	Non-Mycorrhizal	Weakly Mycotrophic	Strongly Mycotrophic	Mycoheterotrophic
Non-Mycorrhizal	Positive	Negative	**Negative**	Positive
Weakly Mycotrophic	Negative	Positive	Positive	**Negative**
Strongly Mycotrophic	**Negative**	Positive	**Positive**	**Negative**
Mycoheterotrophic	**Negative**	Positive	**Positive**	**Negative**

Note: The presence of mycotrophic plants can increase fungal density, while AM fungal density may be decreased due to the presence of nonmycorrhizal and mycoheterotrophic plants. Each column then presents the expected consequences of these changes in AM fungal density on the growth of a second plant species. The bold words indicate greater than expected magnitude of effect.

22.2.3.3 Plant Parasitism of AM Fungi

A specialized group of plants, called mycoheterotrophs, have evolved the ability to derive energy from fungi. These plants can be completely achlorophytic or partially so and have been found to be associated with a range of fungi, including ectomycorrhizal fungi and AM fungi (Leake, 1994; Bidartondo et al., 2002). The growth rates of AM fungi would presumably decrease with increasing density of these plants, and the growth rates of the plants would be expected to increase with increasing densities of AM fungi (Table 22.1), though these effects remain to be demonstrated. The expected pairwise dynamic would be one similar to other antagonistic interactions in which there is negative feedback between abundance of mycoheterotrophs and abundance of AM fungi.

22.2.3.4 Competition between Plants and Mycorrhizal Fungi

Interactions in which both populations have reduced growth rates in the presence of the other are classified as competitive. Competitive interactions can result from depletion of a common resource. Because plants are autotrophs, they are not likely to compete with fungi for carbohydrates (though the existence of mycoheterotrophs causes one to pause). Plant roots and mycorrhizal fungi are likely to compete for access to soil minerals. The competition for mineral resources would cause the net sign of the effect of fungi on plants to depend on the environment. Mineral resource competition, however, is unlikely to cause the sign of the net effect of plants on fungi to become negative because mycorrhizal fungi are still dependent on plants for their carbohydrates. Therefore, resource competition between plants and AM fungi is unlikely to generate a mutually antagonistic interaction. Such mutually antagonistic interactions may result, however, from interference competition, particularly in the interaction between mycorrhizal fungi and some nonmycorrhizal plant species. Plants in the Brassicaceae, which are typically nonmycorrhizal, have been shown to have negative responses to mycorrhizal fungi, and these plants have also been shown to produce allelochemicals that inhibit mycorrhizal fungal hyphal extension and spore germination (Allen et al., 1989; Johnson, 1998; Roberts and Anderson, 2001). As a result, this interaction may fit the classic definition of competition. It remains to be demonstrated that the density of mycorrhizal fungi declines more quickly in the presence of these antagonistic hosts than in the absence of any host plant.

22.3 MECHANISMS OF AM FUNGAL MEDIATION OF PLANT–PLANT INTERACTIONS

AM fungi could mediate plant–plant interactions by modifying resource competition or indirectly through changes in AM fungal density or composition. AM fungi would modify resource competition if their presence modifies the plant's niche (Van der Heijden, 2002; Reynolds et al., 2003). Alternatively, AM fungi could modify competition by facilitating resource sharing between plants (Francis and Read, 1984; Grime et al., 1987). In these two views, the presence of the AM fungi is required, but the modification of competition is not explicitly a function of AM fungal density. Separate from modifying competition, plant–plant interactions could be indirectly affected through changes in AM fungal density or composition (Bever, 2002b; Bever et al., 2002). While these potential mechanisms are not mutually exclusive, they develop different views of mycorrhizal mediation of plant–plant interactions, and we therefore discuss them separately.

22.3.1 Modification of Resource Competition by AM Fungi

Mycorrhizal fungi could modify competitive interactions among plant species by influencing the realization of a plant's abiotic niche. Given the role of mycorrhizal fungi in resource acquisition, it is likely that the presence of mycorrhizal fungi could alter plant nutritional niches (Van der Heijden, 2002; Reynolds et al., 2003). It is also possible that particular fungi can be important for expression of other niche dimensions, including seasonality (Bever et al., 2001). To alter competitive interactions, mycorrhizal fungi would have to differentially affect the niche of competing species. Van der Heijden (2002) identified that the presence of mycorrhizal fungi could alter the resource niche of mycorrhizal plants relative to nonmycorrhizal plants. He imagined a scenario in which the presence of mycorrhizal fungi shifts the resource uptake of mycorrhizal plants in a manner that could lead to dominance of the mycorrhizal plants or to their coexistence with nonmycorrhizal plants.

Mycorrhizal fungi could also differentially shift niches of mycotrophic plant species. In particular, it is possible that different fungal species are necessary for partitioning of belowground resources. AM fungal species have been shown to forage for resources differently (Smith et al., 2000), and different fungal species may provide differential access to soil nutrients. For example, some may be better at foraging for organic phosphorus, while others are better at foraging for mineral phosphorus. In this case, the presence of particular fungi may be required for plant species to occupy different soil nutritional niches (Reynolds et al., 2003). As a result, the interspecific competitive interactions could be modified by AM fungal community composition, with plant species coexistence dependent upon individual plants having their appropriate mycorrhizal fungal symbionts. At present there is little evidence for this scenario, though the observation that overall resource use increased with increasing diversity of AM fungi (Van der Heijden et al., 1998b) is consistent with this mechanism.

Plants can also partition resources across seasons (Fowler and Antonovics, 1981), and it is possible that different species of AM fungi are necessary for the success of cool-season plants vs. the warm-season guild. Indeed, AM fungal species have also been found to differ in their seasonality (Merryweather and Fitter, 1998; Pringle and Bever, 2002). Moreover, within one community, the fungus with the most markedly cool-season phenology grew best with, and was spatially associated with, the plant that shared a markedly cool-season phenology (Bever et al., 1996; Schultz, 1996; Pringle and Bever, 2002).

22.3.2 Carbon Transfer in Common Mycorrhizal Networks

Because of low specificity of association, shared AM fungi could alter plant–plant interactions by providing conduits for sharing resources, such as carbon (Francis and Read, 1984; Grime et al., 1987). This hypothesis has been supported by the observation that labeled carbon fixed by one plant has subsequently been found in greater abundance in a second plant with which it shares mycorrhizal fungi than plants that do not share mycorrhizal fungi. To date, studies of carbon transfer suggest that it is more substantial and common in ectomycorrhizal fungi (Robinson and Fitter, 1999; Simard et al., 2002), but there is also a recent report of transfer to the leaves through AM fungi (Lerat et al., 2002).

It is possible that the carbon transfer between plants via mycorrhizal networks could alter plant–plant interactions. One hypothesis for predicting the direction of these effects is the *source–sink hypothesis.* This hypothesis builds on the physical process of resource flow from plant physiology and views mycorrhizal hyphae as passive conduits (Robinson and Fitter, 1999). Under this hypothesis, carbon is expected to flow from the strongest source to the strongest sink, thereby potentially alleviating interspecific competition through facilitation of the weakest competitor. This prediction was supported by a mesocosm study in which inoculation with mycorrhizal fungi in a mixed community of plants greatly decreased the dominance of the best competitor (Grime et al., 1987).

Several studies specifically designed to test the source–sink hypothesis have given little support for its predictions. For example, while the source–sink hypothesis predicts that the smaller plant will be the net recipient of nutrient transfer, Kytöviita et al. (2003) found that seedlings of four subarctic meadow plant species connected by a common mycorrhizal network to a larger plant were significantly smaller than mycorrhizal seedlings unconnected to a larger plant. This suggests that the common mycorrhizal network facilitated the competitive superiority of the larger plant, thus contradicting the source–sink hypothesis. In separate studies, shading of plants, which would be predicted to increase sink strength, actually decreased transfer of labeled carbon (Hirrel and Gerdemann, 1979). Similarly, clipped plants, which were predicted to be sinks, actually acted as sources of carbon (Waters and Borowicz, 1994).

There is no disputing that there is a net movement of carbon between photosynthetic plants and mycoheterotrophs via mycorrhizal hyphae. Nevertheless, the ecological importance of carbon transfer among photosynthetic plants remains in doubt (Robinson and Fitter, 1999). In the work on the plant–AM fungal interaction in particular, the amount of carbon transferred was very small (Robinson and Fitter, 1999). Even in the ectomycorrhizal system, it remains to be demonstrated that a photosynthetic recipient of carbon receives more carbon from the fungus than it gives that fungus. This is a critical point, as an alternative explanation is that the carbon movement from fungus to plant is incidental to symbiosis establishment or transfer of soil minerals (Smith and Read, 1997). Organic signaling molecules may be transferred from fungus to plant as part of the initiation of the symbiosis, or soil minerals may be transferred to the plant in organic form. In either case, some carbon would move from fungus to plant even while that plant rewards the fungus with a greater amount of energy. As a result, labeled carbon from one plant would show up in a second plant as a consequence of fungal growth from the root of one plant to the root of another (Fitter et al., 1998). In this view, carbon exchange between plants may not reflect a meaningful energetic subsidy of the second plant. It does reflect interactions with the same AM fungi, which can have consequences on plant–plant interactions mediated by changes in fungal density, a perspective developed below. It is interesting to speculate that mycoheterotrophic plants could have evolved by capitalizing on such normal transfers of carbon between mycorrhizal fungi and their hosts.

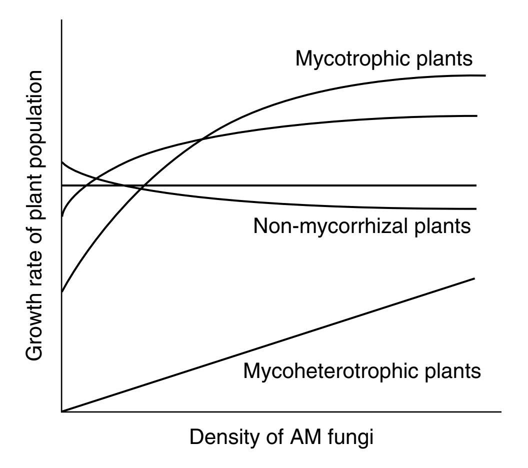


Figure 22.3 The expected relationships between plant population growth rates and density of AM fungi for different types of plants assuming a saturating response and a trade-off in the ability to grow with AM fungi and the ability to grow without AM fungi. The population growth rate of the mycoheterotrophic plant is assumed to increase approximately linearly over the range of densities of AM fungi commonly observed.

22.3.3 Indirect Effects between Plants Mediated by Changes in AM Fungal Density

Interactions between plants may be mediated by changes in the density of shared AM fungi (Figure 22.3). That is, the presence of a given plant type can cause changes in the density of AM fungi, which can then alter the growth of that plant type compared with a competing plant species. Such indirect effects are likely given the low specificity of association where plants will often be interacting with the same fungal population, both positively and negatively. Indeed, the level of infection of plants in the field has been shown to depend on the identity of their neighbor (Jastrow and Miller, 1993). The dynamics resulting from such feedback through changes in fungal density have been explored previously using a general model (Bever et al., 1997; Bever, 2003). Here, we develop specific expectations for interactions between four types of plants identified in Table 22.1. We do this by building up from the modules of pairwise interactions (Table 22.1) in a way that has proven successful in understanding antagonistic interactions (Holt and Lawton, 1994).

The role of AM fungal density in mediating plant–plant interactions depends on the relationship between plant population growth rates and density of AM fungi (Figure 22.2). In light of these relationships, we build on an assumption of a trade-off in growth with and without AM fungi; i.e., plants that grow best with AM fungi grow worst without AM fungi. The existence of this trade-off is well supported, both by empirical observations of plant growth rates (Fitter, 1977; Allen and Allen, 1984) and by the mechanistic observation that plants that are not responsive to AM fungi generally invest greater resources in fine roots and root hairs that allow them to directly acquire soil resources (St. John, 1980; Hetrick et al., 1992; Schultz et al., 2001). Conversely, plants that are more responsive to AM fungi generally have coarser roots and therefore perform poorly in the absence of AM fungi.

The trade-off in plant growth with and without AM fungi translates into different intercepts and maxima in the response of plant population growth rates to AM fungal density, as illustrated for a saturating relationship between plant growth and AM fungal density (Figure 22.3). Nonmycorrhizal plants are assumed to have the highest population

growth rates in the absence of AM fungi, but the lowest growth rates among autotrophic plants in the presence of AM fungi. Similarly, plants with low levels of mycotrophy are expected to have higher population growth rates in the absence of AM fungi than plants with high mycotrophy. That is, the responsiveness of plants to AM fungi is assumed to correlate with the obligacy of the relationship for the plant because of the trade-off in nutrient acquisition.

As mycoheterotrophic plants directly parasitize fungi, they will likely have a qualitatively different response to AM fungal density. Specifically, mycoheterotrophic plants cannot grow in the absence of AM fungi, and their rates of population growth will likely not saturate until a much higher density of AM fungi because their growth will not be limited by light or soil minerals. We represent the population response of mycoheterotrophic plants as approximately linear over the range of densities that AM fungi typically vary (Figure 22.3).

Examination of Figure 22.1 and Figure 22.3 allows prediction of the effect of fungal population dynamics in mediating plant–plant interactions. We address these issues by considering pairwise plant–plant interactions. Specifically, we consider the effect that each plant type has on the density of AM fungi, and then consider whether this change in fungal density will have positive or negative effects on a second plant species (Figure 22.3). The direction and an estimate of the relative strengths of these effects are tabulated in Table 22.1. We infer the long-term dynamics by contrasting the effect of each category of plant on plants in the same category with the effect on plants in other categories as derived in Bever et al. (1997).

22.3.3.1 Interactions with Nonmycorrhizal Plants

The category of nonmycorrhizal plants likely includes species that interact antagonistically with AM fungi and those that have no effect on AM fungi. For nonmycorrhizal species that inhibit the growth of AM fungi, we would expect the decreased density of AM fungi to have indirect positive effects on the growth of other nonmycorrhizal plants. To the extent that nonmycorrhizal plants reduce the density of AM fungi, such plants would also have indirect negative effects on the growth of mycotrophic plants. This indirect inhibition would be small for plants with low mycotrophy, but could be substantial for plants with high levels of mycotrophy (Figure 22.3). This indirect effect could generate a positive feedback dynamic that could reinforce an initial dominance of nonmycorrhizal species and inhibit the establishment of highly mycotrophic species. Alternatively, once mycotrophic plants are established, the density of AM fungi may increase, thereby inhibiting the growth of nonmycorrhizal plant species. These expectations are supported by empirical work in which nonmycorrhizal plants are competitively superior to mycotrophic plants in the absence of mycorrhizal inoculum, but competitively inferior in the presence of mycorrhizal inoculum (Allen and Allen, 1984). Also, positive feedback was observed between nonmycorrhizal introduced plant species and mycotrophic native species by Klironomos (2002), which may have been mediated by changes in AM fungal density.

22.3.3.2 Interactions between Ecto- and Arbuscular Mycorrhizal Plants

A similar positive feedback dynamic might be expected between plants that are dependent on AM fungi and plants dependent on ectomycorrhizal fungi. Dominance by ectomycorrhizal plants could reduce the density of AM fungi, thereby reducing the success of plants that are dependent on AM fungi. An experimental test of this possibility found no reduction in AM fungal inoculum density under an ectomycorrhizal canopy (Lovelock and Miller,

2002). The AM fungi under the oak, however, was less effective at promoting growth of an AM mycotrophic seedling (Lovelock and Miller, 2002). The flip side of this dynamic has been observed, where ectomycorrhizal seedlings enjoy inoculum potential and greater growth in proximity to ectomycorrhizal canopy trees (Dickie et al., 2002).

22.3.3.3 Interactions between Mycotrophic Plants

Given the trade-offs between growth with and without mycorrhizal fungi represented in Figure 22.3, we would expect that weakly mycotrophic species would have the highest population growth rates at low density of mycorrhizal fungi, while strongly mycotrophic species would have the highest population growth rates at high densities of AM fungi. This generates the prediction that the highly mycotrophic plant species would benefit the most in mixed-plant communities from the presence of mycorrhizal fungi. This *mycotrophic hypothesis* is well supported empirically and offers a very credible alternative explanation to attempts to explain the effect of AM fungi on plant–plant interactions through carbon transfer (Bergelson and Crawley, 1988). Several studies have found that the most mycotrophic plant species benefited the most in mixture from mycorrhizal inoculation (Grime et al., 1987; Van der Heijden et al., 1998b; Hartnett and Wilson, 1999).

All mycotrophic plants are expected to serve as capable hosts of AM fungi, with the highly mycotrophic species likely the better hosts (as argued above). As a result, all mycotrophic plants have the potential to increase the density of AM fungi and thereby facilitate each other's growth (Table 22.1). The most mycotrophic plants are expected to benefit the most from this feedback through change in density, potentially making the most mycotrophic plant species the net beneficiaries of AM fungal density dynamics, as has been hypothesized in discussions of the role of mycorrhizal dynamics in succession (Janos, 1980; Reynolds et al., 2003). In a test of feedback through changes in AM fungal community, the density of AM fungi did reach highest densities in association with the most mycotrophic plant species, *Allium vineale.* However, *Allium* did not grow best with its own fungal community as predicted (Bever, 2002a), perhaps because of confounding changes in the composition of the AM fungal community, as discussed below.

22.3.3.4 Interactions with Mycoheterotrophic Plants

By supporting growth of AM fungi, mycotrophic plants indirectly facilitate the growth of mycoheterotrophic plants. Conversely, by reducing the population growth rates of AM fungi, mycoheterotrophic plants indirectly inhibit the growth of mycotrophic plant species. This effect may be small; however, if the relationship between mycotrophic plant growth and AM fungal density is best described as a plateaued function (Figure 22.2 and Figure 22.3), then the net interaction is one in which mycoheterotrophic plants indirectly parasitize mycotrophic plants through their effects on densities of AM fungal populations (Leake, 1994). Mycoheterotrophic plants are expected to exert negative density dependence on their own rates of population growth through their negative effects on the density of their AM fungal resource. This negative density dependence could regulate the population size of mycoheterotrophic plants.

To the extent that mycoheterotrophic plants decrease AM fungal population density, they will indirectly facilitate the growth of nonmycorrhizal plant species. The success of mycoheterotrophic plant species could then create ecological opportunities for nonmycorrhizal plant species in areas otherwise dominated by mycotrophic plants. Conversely, to the extent that nonmycorrhizal plant species decrease AM fungal densities, they indirectly inhibit the growth of mycoheterotrophic plant species.

22.3.4 Indirect Effects between Plants Mediated by Changes in AM Fungal Composition

In much of the discussion of the impact of the presence and change in density of mycorrhizal fungi above, the AM fungal community is simplified into a homogeneous population. However, there is accumulating evidence that AM fungal communities are diverse and that individual fungal species are ecologically distinct. For example, we have found 37 species of AM fungi coexisting within a single old field (Bever et al., 2001), and molecular characterization of communities suggest that this level of diversity is not unusual (Helgason et al., 2002; Husband et al., 2002). Because these AM fungal species also differ in ecologically important ways, including their response to environmental gradients, average growth promotion, and specificity of growth promotion, the composition and dynamics of communities of AM fungi can also mediate plant–plant interactions (Fitter, 2000; Bever et al., 2001). In the next section, we develop different ways in which this mediation can happen, focusing exclusively on mycotrophic plant species.

22.3.4.1 Change in Overall Effectiveness of AM Fungal Community

Variation in the average effectiveness of growth promotion of the AM fungal community could alter plant–plant interactions in a similar fashion as changes in overall AM fungal density. An increase in the average effectiveness of the AM fungal community would benefit all mycotrophic plant species at the expense of nonmycorrhizal plants, and it would also benefit highly mycotrophic plants more than less mycotrophic plants. If the most mycotrophic species also promotes the growth of the most effective AM fungal isolates, then this could generate a positive feedback, reinforcing the abundance of the most mycotrophic plant species and the most effective AM fungal species.

22.3.4.2 Host Specificity in AM Fungal Growth Promotion

The fact that individual species of AM fungi vary in their host specificity of plant growth promotion has several important consequences for plant–plant interactions. At the most basic level, the success of a given plant species may depend on the abundance of a particular fungal species, while the success of a second plant species may depend on the abundance of a second plant species. Evidence for this level of specificity has been found in several systems (Adjoud et al., 1996; Van der Heijden et al., 1998a, 1998b; Bever, 2002b; Helgason et al., 2002). Therefore, the composition of the AM fungal community could alter plant–plant interactions.

In this scenario, the long-term dynamics of the plant community would depend on the dynamics of the AM fungal community. If the composition of the AM fungal community is spatially heterogeneous, then the composition of the AM fungal community would remain an important source of environmental heterogeneity for plants and a potential determinant of plant–plant interactions. Fine-scale spatial heterogeneity in the AM fungal community composition is well documented and is correlated with both plant and environmental parameters (Bever et al., 1996; Schultz, 1996; Helgason et al., 2002; Lovelock et al., 2003). If the distribution of individual species of AM fungi is stochastic or is determined by environmental factors, then AM fungal community composition could alter the outcome of plant–plant interactions, with the spatial variation possibly contributing to the maintenance of diversity in the plant community.

However, if the distribution and abundance of AM fungal species respond to local plant species composition, as noted above and as expected from the evidence that the relative population growth rates of AM fungi are host species specific, then the role of

AM fungi in plant–plant interaction will be determined by feedback through changes in the AM fungal community composition (Bever, 1999; Bever et al., 2002). AM fungal community feedbacks can be positive or negative, and these feedbacks will generate frequency dependence in the outcome of plant–plant interactions.

Positive feedback results from symmetry in fitness relationships in which the fungus that promotes the growth of a given plant is also the fungus that has the highest growth rate on that plant host. As a result, an initially high frequency of one plant type will result in an increase in abundance of its preferred fungus, which thereby increases the plant's growth rate relative to that of other plants (Bever, 1999; Bever et al., 2002). This positive feedback generates positive frequency dependence between plant species, in which the most common plant species inhibits the growth of the less common plant through changes in the AM fungal community. The ultimate outcome of this dynamic is the exclusion of the less common species, at least on a local scale. This dynamic was suggested by the results of Klironomos (2002), though it is also possible that the positive feedbacks observed in this study were mediated by changes in AM fungal density (as discussed above) rather than composition. Other evidence for positive feedback comes from work on frequency dependence between genotypes of *Allium vineale*. Ronsheim (1996) found that *Allium* genotypes grew better when planted near neighbors of the same genotype. The positive frequency dependence could result from AM fungal community dynamics, as supported by the observation of positive soil community feedback between *Allium* genotypes (Bever et al., 1997) and from factorial manipulations of the soil community and neighbors (Ronsheim and Anderson, 2001).

Alternatively, the dynamics between plants and fungi may be characterized by negative feedback. In this case, the presence of one plant can facilitate the growth of a second plant species through changes in the composition of the AM fungal community (Bever, 1999; Bever et al., 2002). This dynamic results from highly asymmetric fitness relations in which the fungus that promotes the growth of a given plant has the highest growth rate on a second plant species. As a result, the AM fungal community dynamic will generate negative frequency dependence in plant–plant interactions and, thereby, will contribute directly to coexistence of competing species. Testing such feedbacks is made difficult by the potentially confounding effects of accumulation of host-specific pathogens (Bever, 2002a; Bever et al., 2002). In a study that eliminated pathogens, we found evidence of negative feedback mediated by changes in the AM fungal community composition between two co-occuring plant species. Specifically, we found that the AM fungus *Scutellospora calospora* accumulated in association with the plant *Plantago lanceolata.* However, *Plantago* grew best with two other species of fungi, *Archaeospora trappei* and *Ac. morrowiae* (Figure 22.2), and these fungi accumulated under *Panicum* (Bever, 2002b). As a result, the presence of *Panicum* caused a change in the composition of the AM fungal community that facilitates growth of *Plantago*. Evidence of a similar dynamic was also found between co-occurring plant species in native grasslands in the eastern U.S. (Castelli and Casper, 2003). How common this dynamic is and what prevents this dynamic from degrading the mycorrhizal mutualism remain to be investigated.

22.4 COMPARING AND CONTRASTING THE MECHANISMS

Mechanisms identified above differ in their long-term predictions for the effect of AM fungi on plant community dynamics. Several mechanisms could facilitate the coexistence of competing plant species, including differential effects on resource utilization, the sharing

of resources, and negative feedback through changes in AM fungal density or composition. Alternatively, positive feedback through changes in the AM fungal density or composition could decrease the likelihood of coexistence of competing plant species. In fact, mycorrhizal fungi have been found to have both positive (Grime et al., 1987; Gange et al., 1990; Van der Heijden et al., 1998b) and negative (Hartnett and Wilson, 1999; O'Connor et al., 2002) effects on plant diversity. Measures of plant diversity have also been found to respond positively to manipulation of the number of AM fungi (Van der Heijden et al., 1998b). This result could be generated by several of the mechanisms through which mycorrhizal fungi may mediate plant–plant interactions, including AM fungal enabling of resource partitioning and negative feedback through changes in AM fungal community composition.

Mechanisms through which AM fungi mediate plant–plant interactions are not mutually exclusive. Rather, we might expect them to act simultaneously. For example, if the presence of AM fungi modifies the interspecific competitive ability of plants through changing the resource utilization of plant species, then it is likely that there are also important effects mediated by changes in mycorrhizal fungal density or composition. The joint effects of interspecific competition and soil community feedbacks have been explored theoretically (Bever, 2003), identifying conditions in which the ultimate dynamics can be determined by either force. In this analysis, the nature of competition was not changing with changing density or composition in the fungal community. Simultaneous effects of AM fungi in modifying resource competition and indirectly affecting plant growth through changes in density have not been investigated. One can imagine that competing best-matching plant and fungal pairs, which would otherwise lead to exclusion, could be stabilized and coexist if the AM fungi contributed to the partitioning of resources between the plant species (Bever et al., 2001).

Data do not currently exist to allow identification of the relative importance of the mechanisms that may mediate plant–plant interactions. The modification of a plant's fundamental niche seems to be a particularly likely mechanism through which AM fungi could modify interspecific competition. A definitive test that this mechanism mediates plant–plant interactions remains to be done. While experimental measures support carbon transfer between plants via mycorrhizal fungi, available data do not suggest that carbon transfer modifies plant–plant interactions. In contrast, there is relatively strong support for the hypothesis of modification of plant–plant interactions through changes in AM fungal density, particularly in successional contexts (Janos, 1980; Medve, 1984; Gange et al., 1990). Negative feedbacks through changes in AM fungal composition have also been demonstrated (Bever, 2002b; Castelli and Casper, 2003), though their relative importance in determining plant–plant interactions remains to be demonstrated. It is our hope that the development and delineation of the potential mechanisms of AM fungal mediation of plant–plant interactions in this chapter will encourage further experimentation that will allow differentiation and substantiation of these processes.

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23

Impacts of Plant Pathogenic Fungi on Plant Communities

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23.1 INTRODUCTION

Pathogens affect plant communities in many ways, with widely different consequences (including negligible) and across vast ranges of spatial (cellular to landscape) and temporal (from the contemporary plant generation to evolutionary time) scales. Plant pathogenic fungi are ubiquitous in plant communities, and their impacts are diverse and often profound. They influence productivity, species composition and diversity, physical structure of communities and their genetic diversity, succession, and stability. Disease is a defining feature of some plant communities. At the same time, fungus–plant interactions are often so complex and subtle as to be nearly invisible. That any given plant community represents a dynamic interplay, usually over a long period, between plants, pathogens, and their environment is often not apparent unless the equilibrium is altered by a perturbation such as a novel pathogen, novel host plant, or an abrupt change in the environment. The effect of fungal pathogens on plant communities is obvious when it is catastrophic, for example, when a new pathogen such as *Cryphonectria parasitica* (Anagnostakis, 1987) or *Cronartium ribicola* (Maloy, 1997) is introduced to an ecosystem dominated by susceptible hosts, or when a susceptible host is grown in an environment more favorable to its pathogens, such as *Mycosphaerella pini* and *Cyclaneusma minus* on *Pinus radiata* in New Zealand (Gibson, 1972; Gadgil, 1984). But such examples are scant compared with the known diversity of plant-associated fungi and suggest that a more widespread and subtle dynamic exists between most plants and pathogens. In this chapter we (1) look to plant pathology for insights into the ecological implications of plant pathogenesis, (2) review the impacts

of invasive pathogens on plant communities as a source of hypotheses and tests of hypotheses on the roles of indigenous pathogens, and (3) illustrate the range and complexity of possible ecological interactions with observations from the forest ecosystems of western North America.

An empirical/experimental approach to ascertaining effects of plant pathogens on communities is not practical for most real-world plant communities because of their complexity. But the ways that pathogens affect the structure and function of plant communities can be inferred from observation or extrapolated from experiments on individual species in simplified ecosystems. Predicting the ecological outcome of any plant–pathogen interaction requires specific knowledge of the host and pathogen life history strategies, the environment in which they are interacting, and the successional, as well as the evolutionary, history of their interactions. This reality of specificity and complexity interferes mightily with the overarching goal of biological science to discover basic principles that allow prediction of future outcomes. Even as we recognize the difficulty of predicting ecological outcomes, new and altered roles of pathogens in ecosystems arising from global climate change, coupled with the ever-increasing spread of invasive species, are increasing the urgency to do so. Understanding the multiplicity of interactions and impacts between hosts, pathogens, and ecosystems sufficiently to predict specific outcomes presents a formidable challenge.

The interplay between plants and pathogens is an evolutionary process, and it takes an evolutionary perspective to appreciate the full range of effects that plant pathogens have on the communities they inhabit. It seems likely that the community structures we describe today are the result of a long process of coadaptation. What we see today is the consequence of disease interacting with other selective forces accumulated across the millenia. Plants have responded to pathogens through evolution of resistance mechanisms and through adjustments to their range. It follows that plant communities have altered over evolutionary time in part due to selective action of pathogens. The role of indigenous pathogens in natural plant communities is often regulatory and may be exercised without dramatic disease. The reproductive fitness of both host and pathogen populations is not threatened by the interaction, so long as the relevant environment varies only within the range to which the players are adapted.

Plant pathologists understand the complexity of host–pathogen interactions. Both the first (Dinoor and Eshed, 1984) and the most recent (Gilbert, 2002) reviews of diseases in natural ecosystems appeared in the *Annual Review of Phytopathology.* Until recently, however, the pathologist's brand of applied plant ecology has been largely excluded from mainstream ecological thinking. Indeed, the plant pathologist's narrower focus on agronomic crops has fed a perception that plant disease is somehow unique to, or even a consequence of, plant stress and monocultural agroecosystems.

J.L. Harper's 1977 book is generally credited with recognition and incorporation of the roles of plant diseases in natural plant populations and communities, and ecological awareness as well as experimental attention have gradually increased since its publication. The careers and writings of Jeremy Burdon, Helen Alexander, and Janis Antonovics (i.e., Alexander, 1992; Burdon, 1993; Antonovics et al., 1996; Holah and Alexander, 1999; Thrall and Burdon, 2003) have been central to the emerging appreciation of the importance of pathogens as determinants of natural plant community structure and process. Recognition of the roles of pathogens in ecosystems in mainstream ecology is marked by the 2003 annual meeting of the Ecological Society of America symposium on "Plant Pathogens in Nature: Rethinking Vegetation Dynamics." The recent review by Gilbert (2002) summarizes experimental and theoretical research in the field to date.

23.2 PATHOGENESIS AS A LIFE HISTORY STRATEGY

Recent appreciation of pathogens in ecological processes is not often reflected in general introductory courses in ecology. Textbook treatments of ecosystem dynamics often include a graphic model of a food chain, featuring primary producers (plants), primary consumers (deer and rabbits), and a food pyramid culminating in an eagle or a grizzly bear. Fungi, if mentioned at all, will appear in a "decomposer" box. Plant pathogenic fungi are seldom mentioned in an ecological context, and even when they are, they are relegated to the "primary consumer" or "herbivore" box. Fungi, even pathogenic fungi, do not neatly fit into any single functional group. They are all heterotrophic to be sure, but they encompass a wide array of life history and nutritional strategies. As a consequence of their functional diversity, they have a wide range of impacts on the plants on which they depend and a corresponding array of community and ecosystem effects.

Broad scientific acceptance of microorganisms as the cause of disease in humans and plants came only in the mid-1800s. It took determined experimentation and brave demonstrations by Pasteur, Koch, DeBary, Léveillé, and colleagues to convince their contemporaries that microorganisms did not arise by spontaneous generation, but that fungi and bacteria were the cause — not the consequence — of decay and disease. Today we understand that pathogenesis is an evolved lifestyle in many fungi. It has arisen (and apparently been lost) repeatedly, being found in branches of all of the major groups of fungi (here including the kingdom Mycota and the unrelated oomycetes). Pathogenicity, the ability to cause disease, is nearly always a consequence of *parasitism*, defined simply as a nutritional dependence of one organism on another, a life history strategy that fungi have explored with considerable evolutionary success.

The pathogenic fungi exhibit the full array of life history and reproductive strategies of fungi in general. Thalli of pathogenic fungi range in size from one or a few cells, such as pathogenic yeasts that specialize on flower parts, to subterranean hyphal networks that span hectares of forest area and whose biomass is estimated in tonnes. Reproductive cycles of many are measured in days or weeks, while some wood-decomposing basidiomycetes may go decades from infection to sporulation. Some increase only belowground via hyphal extension on roots, others are vectored by insects or carried passively in flowing water, and still others form airborne spores, more or less resistant to dessication.

Fungi are characterized by absorptive heterotrophic nutrition and a filamentous form. Most free-living fungi are saprobes, decomposing dead organic material for their own nutritional needs. However symbiosis, including parasitism, apparently has been a strong selective force in fungal speciation. As a consequence of a parasitic habit, numerous fungus species have acquired a competitive advantage through pathogenesis — the ability to cause disease. By extracting nutrients from living plants, fungal parasites may grow and reproduce free of the intense competition for resources among saprotrophs.

To appreciate pathogens in the ecological sense, it helps to consider their evolutionary strategies. Parasitic fungi have found an evolutionary advantage in the fierce competition for substrate and survival — they have evolved a wide array of mechanisms to colonize organic matter while it is still living and unavailable to the (usually) numerically superior saprobic microorganisms. Some colonize foliage and others the roots, some the xylem and others only parenchyma tissue. Some exude toxins that disrupt membranes and then absorb the nutrients that leak out; others break down the cellulose of dead xylem heartwood cells into simple sugars while avoiding the living shell of the tree. Some simply get a head start on strict saprotrophs by colonizing tissues just before they senesce, e.g., certain endophytes or hemibiotrophs (Luttrell, 1974), but others find their advantage in

the most vigorous plants or tissues. Some have developed intimate partnerships, over evolutionary time, with insects and other organisms that play key roles in epidemiology and pathogenesis. Different strategies of pathogenesis have different consequences to host population structure and community diversity.

The plant hosts of parasitic fungi also span the entire evolutionary diversity of the kingdom and exhibit an array of life histories as diverse as the fungi. They all share a structure based on cellulose, however, and an energy metabolism based on hydrolysis and oxidation of sugar. Plant parasitic fungi have evolved mechanisms to tap those sources. The advantage in any arms race would seem to favor the fungi, with their genetic plasticity and shorter generation times. Clearly, plant evolutionary survival depends on successful protection from pathogens. In fact, most plant species are resistant to most species of pathogenic fungi. Generalist fungal parasites, species that can successfully parasitize a wide range of host species, are comparatively rare. Combinations of constitutive physical and chemical barriers block the development of all pathogens except the few that have evolved specific "keys" to bypass or overcome host barriers. But such adaptations come at a cost, and particular adaptations often irreversibly affect the course of future evolution. Even with a key, the pathogen is often slowed or confined to particular host tissues by host defenses, and may be temporarily thwarted when the host changes the lock through evolutionary selection for genes for resistance. This dynamic interaction appears to favor increasing specialization in the fungal parasites, leading to increasing reproductive isolation and speciation. Susceptibility is the rare exception in the broad range of host–pathogen interactions. There are some generalists among the pathogens, able to cause disease on many unrelated plant species, but these tend to be opportunists active on stressed or senescent plants, or sugar fungi, able to disrupt the unlignified primary tissues of seedlings. Even susceptible reactions are heavily regulated by the environment.

23.3 THE PLANT DISEASE TRIANGLE

Disease is the consequence of the interaction of a virulent pathogen genotype and susceptible host genotype in a conducive environment. A spore of an aggressive pathogen may land on the leaf of a susceptible plant genotype, but no disease will ensue unless the environmental conditions on the leaf surface allow spore germination, and still, the other microorganisms already present on the leaf may interfere with infections through various competitive interactions. The resulting infection of one plant individual may be sufficient to ensure the evolutionary survival of the fungus because of its powers of reproduction, but it is unlikely to affect the survival of the plant species because evolution acts on populations, not individuals. And unless the individual plant is killed by the pathogen, and that individual held a dominant position in the plant community, the impact on community structure or function will be small. However, because fungi have relatively short life cycles, and often reproduce prolifically, there is the potential for spread of disease through time and through a host population — an epidemic. The dimension of time converts the disease triangle to a tetrahedron and amplifies the opportunities for impact on the larger plant community.

To understand the ecological impacts of plant pathogens, it is necessary to extend the timescale to encompass evolutionary interactions between plants and their pathogens. The significance of any diseases that we might observe at one point in time in a forest or grassland community is the product of not only the particular species of plant and pathogen that happen to be present, but also the overall climate and the recent weather. It is also conditioned by the coevolutionary (broadly defined) history of the organisms. We often

observe détente, but can no longer see the generations of behavioral, morphological, and biochemical changes that allow the modern coexistence. Exotics provide examples, at least, of pathogenic potential.

23.4 NATIVES AND EXOTICS

The best-known and most dramatic examples of pathogen effects on natural plant ecosystems involve recently introduced invasive pathogens. The frequently cited examples include *Cryphonectria parasitica* (chestnut blight) and *Cronartium ribicola* (white pine blister rust) in North American forests and *Phytophthora cinnamomi* in Australian forests and heathlands, but there are many others (see Chapter 43). Exotic pathogens are of great ecological concern because of the rapid change they can force on susceptible plant communities. Their impacts differ from indigenous pathogens in the magnitude and rapidity of ecological change they can trigger. They provide a reminder of the powerful evolutionary forces that pathogens have exerted on plants. They also graphically illustrate the extremely varied impacts that even closely related organisms can have on community structure and dynamics, and the challenges facing ecologists to generalize and predict (Hansen, 1999).

Consider the invasive pathogens *Phytophthora cinnamomi* and *Phytophthora lateralis*. Both are soilborne and water dispersed, but they have had dramatically different impacts on affected plant communities. The reduction in plant species diversity caused by *P. cinnamomi* in the extremely diverse flora of the Jarrah eucalyptus forest and heathlands of Western Australia (Shearer and Dillon, 1995, 1996) and different eucalyptus communities in Australia's Victoria state (Weste, 1981) is well documented. Here a broad-host-ranging pathogen has met a diverse but unusually uniformly susceptible plant population. The *Eucalyptus* species are relatively more resistant than much of the understory, but inoculum increases on highly susceptible species until even the trees are killed. With loss of the overstory, microclimatic and soil water relations are changed, leading in some areas to the virtual desertification of the landscape. But even in Western Australia, the consequences of the introduction vary from site to site, depending on soil and rainfall, as they affect reproduction of the pathogen, and on the susceptibility of the plant association.

By contrast, in much of the shortleaf pine (*Pinus echinata* Mill.) forest of the southeastern U.S., *P. cinnamomi* has triggered an increase in forest species diversity. In this region, shortleaf pine colonized and dominated abandoned agricultural lands. Under conducive soil conditions, *P. cinnamomi* kills the fine rootlets on this tree. As the early seral stands matured and came into intertree competition for scarce resources on the poorly drained and eroded soil, the effects of *P. cinnamomi* became more pronounced in the continual cycle of rootlet mortality and rootlet regeneration, triggering little-leaf disease and slowly killing the pines (Campbell and Copeland, 1954). The diseased pines were gradually replaced by a more diverse and disease-resistant late successional hardwood forest, which has in turn ameliorated the soils and made conditions less favorable for the pathogen (Tainter, 1997).

The dramatic impacts that *P. cinnamomi* has had around the world are in part a consequence of its very broad host range. *P. lateralis*, on the other hand, kills only *Chamaecyparis lawsoniana* (Murr.) Parl. (Port-Orford-cedar, or POC) and a few *Taxus brevifolia* Nutt. (Pacific yew) trees where it has been introduced in the Pacific Northwest of North America (Murray and Hansen, 1997; Hansen et al., 2000a). The pathogen has spread throughout the limited native range of its cedar host and losses continue. POC tolerates the ultramafic soils scattered throughout the region and is commonly found in association with rare plant species. In the southern part of its range, POC is found primarily

along streams and areas with year-round seepage. In the north, POC commonly grows mixed with other conifers, in upland as well as riparian areas. POC growing along streams is especially vulnerable to *P. lateralis*. Essentially all trees growing with roots in contact with normal winter high-water flows are killed within a few years of introduction of the pathogen to the stream.

The immediate impact of this exotic, host-specific pathogen is to reduce species richness by one or two tree species in those parts of a stand that are exposed to zoospores in water or chlamydospores in transported mud from vehicles. Along streams, especially in the southern part of its range, cedar may be the dominant riparian tree, and its death can have far-reaching impacts on both streamside plant communities and aquatic ecology. Further north in mixed-species stands, where POC is usually a minor component, loss of cedar is less significant, as the crowns of other conifers expand to take its place; often there is little or no change in the plant communities of the understory. On ultramafic soils where cedar may be the principal, or indeed the only, tree species, however, the consequences to stand structure may be dramatic. Even here, though, the change in overall plant diversity may be slight because soil chemistry and water availability, not overstory canopy and light, are apparently the main factors regulating what species will grow.

Today, the varying impacts of *P. lateralis* on forest community structure are dramatically marked by dead and dying cedar trees. What would an observer deduce years from now if the federal programs to plant resistant POC selections and limit further spread of the pathogen are abandoned and forgotten (Anonymous, 2004)? POC will still be part of the forest community in many places, but it will no longer be important in riparian areas. Will *P. lateralis* still be recognized as the cause of the new forest structure?

23.5 EFFECTS OF INDIGINOUS PATHOGENS ON NATURAL PLANT COMMUNITIES: EXAMPLES FROM WESTERN CONIFEROUS FORESTS

By causing disease, plant pathogenic fungi can regulate many aspects of community structure and function. In western forests, some pathogens alter life expectancy and reproductive success, species composition and stature, nutrient cycling, and primary productivity. They change local population structure of individual species and landscape scale patterns of plant succession. Some pathogens play critical roles in determining range limits and habitat occupancy. Many of these changes alter the diversity of ecosystems, sometimes in unexpected ways (Hansen and Goheen, 2000). In order to understand and predict the ecological impacts of pathogens in a particular community, some understanding of the basic functioning of the ecosystem is necessary.

Forests are naturally dynamic communities of organisms, with trees growing old, competing, dying and being killed, and being replaced by other trees, often of other species. Understory vegetation changes in response to changes in the overstory. The natural process of succession is accompanied by dramatic changes in plant diversity. Succession in the Douglas-fir forests in the western slopes of the Cascade Mountains in western North America illustrates the kinds of changes that occur through time (Spies, 1997). In this region stand-replacing wildfire, or its modern surrogate, clear-cut harvesting, controls the overall pattern of succession. The natural fire return interval averages between 300 and 400 years in these Mediterranean climate forests. Immediately following a stand-replacing fire, local plant species diversity is near zero, but it increases rapidly in the following years as herbaceous plants and shrubs colonize the open ground. Plant species diversity is usually at its highest point in these early seral communities (Long, 1977). With time (varying by

distance to a seed source) Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) colonizes the site and grows up through the low herbaceous and shrub vegetation, often forming dense, nearly even-aged stands. As the crowns of the trees expand and meet, light penetration to the forest floor is gradually reduced, and the lower vegetation dies or retreats to gaps in the canopy. Diversity decreases and stays low for 100 years or more, depending on intermediate disturbances. The light-demanding Douglas-fir is the early seral dominant species, and its potential life span is much greater than the usual fire return interval. Relatively shade tolerant trees such as western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) are the late seral, or potential climax trees, in these forests. Hemlock seedlings are established beneath the Douglas-fir, but they are confined to the understory until some disturbance (usually a pathogen) kills the fir and allows in enough light to stimulate height growth. As mortality of the fir increases with age, plant diversity slowly increases in the light gaps, but even in old-growth forests it usually does not reach the species richness present shortly after fire or harvest.

Pathogens that kill trees can change the forest. The results may be evident from the canopy down to the forest floor. Chestnut blight and white pine blister rust, destructive introduced pathogens in North America, transformed forests (Chapter 43). A number of indigenous pathogens also kill trees. Some are opportunistic, or facultative parasites, able to live saprobically or to colonize weakened hosts, such as the root-rot fungi *Poria subacida* and some species of *Armillaria*. In the Douglas-fir forest, these pathogens kill scattered, slower-growing trees that are being overtopped by neighbors during the intensive competition of the stem exclusion stage of stand development. Resources and height growth are thus concentrated on the more vigorous survivors.

Other decay fungi that are not directly lethal may change the fitness of infected trees by increasing the probability of breakage. In young forests, *Fomitopsis cajanderi* infects Douglas-firs that suffer broken tops from snow, ice, or wind damage. It causes a central column of brown-rot decay. Later windstorms are much more likely to break these decayed trees than adjacent sound trees. Interestingly, in old forests *Fomitopsis officinalis* instead of *F. cajanderi* infects trees with broken tops or lost limbs. *Phaeolus schweinitzii* causes a root and butt rot in several conifer species, including Douglas-fir. Large infected trees are usually not killed directly by the pathogen, but do have increased chances of breakage in storms. In one study in mature timber in the central Oregon Cascades, the overall incidence of *P. schweinitzii*, as evidenced by butt rot on cut stumps in clear-cuts, was about 15%. In adjacent uncut stands, however, about 85% of the windthrown or collapsed trees resulting from winter storms failed as a result of butt rot (Hansen and Goheen, 2000). In each of these examples, the distribution of diseased trees, and subsequent mortality, is scattered. Single trees are killed, and the crowns of neighbors expand, closing the gap. The transitory increase in light below the canopy may allow accelerated growth of established understory trees.

Death of groups of trees resulting in gaps in the canopy allows increasing diversity as the Douglas-fir forest ages. Many agents kill trees and thus affect diversity, and ecologists often lump pathogens with hurricanes, lightning, and fire as disturbance agents. Gap or patch dynamics is an important component in current studies of forest ecosystem dynamics (Pickett and White, 1985), with gaps in the canopy created by disturbance agents. Pathogens differ from other disturbance agents in at least two fundamental ways, however, and pathogen-induced gaps have different consequences to forest communities than other types of gaps. Pathogens usually affect species differentially; that is, they exhibit host specificity, and pathogens act slowly. A lightning strike or a tornado kills all the tree species in a discrete patch instantaneously, but laminated root rot, for example, kills Douglas-fir but not western hemlock in lowland forests of the Pacific Northwest, in a patch that slowly

increases in size throughout the life of the stand (Hansen and Goheen, 2000). Pathogens also interact with other disturbance agents in the forest so that mortality may increase in pulses. Trees structurally and physiologically weakened by root rot are more vulnerable to windthrow and insect attack. Small patches of mortality commonly ascribed to wind or the Douglas-fir bark beetle are usually centered on root-rot foci (Goheen and Hansen, 1993).

The death of one tree or a slowly increasing group of trees of the same species immediately reduces the local species richness by one, but endemic pathogens that kill trees seldom have a direct effect on species richness at the landscape or even the stand level. Diversity measured by the indices that include species evenness, however, may actually increase on a stand or landscape scale as the result of either native or introduced pathogens. Greater diversity may result when a dominant species is killed, increasing the proportion of otherwise minor species in the stand (Holah et al., 1993).

In long-lived forests, the indirect effects of mortality on diversity are usually greater than the direct loss of individual trees. When a tree dies, it frees resources for other plants, and the environment of the gap may favor growth of other species. In time, the plant community adjusts to the new circumstances and may be either more or less diverse after disturbance than before. The outcome depends on both the pathogen and the forest community (Holah et al., 1993, 1997). If the pathogen kills only a single tree species, the consequences are different from a more omnivorous pathogen. Pathogens that spread by aerial spores and kill scattered single trees in the stand have a very different effect than pathogens that spread vegetatively and kill slowly expanding groups of trees. The consequences of tree mortality are different in mixed-species stands than in natural or planted monocultures, and in early successional stands than in late successional forests. Local outcomes are strongly influenced by the chance of seed availability. Generalizations are difficult given the many possible combinations of circumstances represented by the forested ecosystems of the west.

Phellinus weirii (Murr.) Gilbertson, cause of laminated root rot of Douglas-fir and other conifers in western North America (Childs, 1963; Buckland et al., 1954), is the principal natural disturbance agent in these forests in the long intervals between stand-replacing wildfires. It spreads from tree to tree as ectotrophic mycelium on roots where they grow in contact, forming slowly expanding mortality centers (gaps) in the stand. Infection centers occupy from 5 to 10% of the Douglas-fir forest area in the west (Goheen and Hansen, 1993). *P. weirii* survives the time span after fire or harvest as mycelium in old decayed roots, starting to spread again when roots of a new generation of susceptible trees grow in contact with the old inoculum.

P. weirii is a major determinant of the structure, composition, and function of natural as well as managed forests in the region (Schowalter et al., 1997). Not surprisingly, it also changes plant community diversity. The direction and magnitude of change depends, however, on local conditions. Mountain hemlock (*Tsuga mertensiana* [Bong.] Carr.) is a mid to late seral conifer at higher elevations in the Cascade Mountains and is very susceptible to *P. weirii*. It gradually replaces the early seral lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) that establishes after stand-replacing wildfire, eventually forming extensive homogeneous stands with scattered remnant pines (Dickman and Cook, 1989). As the mountain hemlock overstory is killed by *P. weirii*, the expanding gaps contain old pines that survived the root rot and early seral herbaceous, shrub, and tree species that can reproduce in the light of the mortality center. Mountain hemlock also reproduces and is eventually killed by the fungus. Species diversity is significantly increased in the gaps and at the stand level by the action of laminated root rot (McCauley and Cook, 1980).

In Douglas-fir forests, by contrast, the impacts of laminated root rot on local species diversity are not so predictable (Holah et al., 1993, 1997; Ingersoll et al., 1996). In old-

growth Douglas-fir forests, where seed sources or advance regeneration of late successional tree species such as western hemlock (*Tsuga heterophylla*) or western red cedar (*Thuja plicata* Donn) are often available, succession may be advanced as these shade-tolerant trees assume dominance behind the mortality front. Douglas-fir is not able to reproduce because there is insufficient soil disturbance and light in the slowly expanding gaps. The result is often a decrease in local species diversity because hemlock and cedar form very dense, dark canopies, allowing fewer understory species to grow than are present beneath a Douglas-fir overstory. In forests where hemlock and cedar are not available to colonize the gaps, diversity may be increased or unchanged, depending on the mix of shrub and herbaceous species that can take advantage of the light. Although the specific consequences differ depending on the species composition and seral stage of the forest, laminated root rot is a major factor in shaping forest structure, composition, and process wherever it is found.

Pathogens that do not kill trees also affect plant communities, but in more subtle ways. Foliar pathogens seldom kill mature trees, although they may kill seedlings and thus affect reproductive success. They do affect growth rate of mature trees, and this in turn can affect the competitive fitness of the tree. A contemporary example of a foliar pathogen affecting forest species composition comes from *Phaeocryptopus gaeumannii* (Rohde) Petrak, cause of the disease Swiss needle cast on Douglas-fir. It is currently forcing changes in forest management practices in coastal Oregon. As with many other pathogens, however, its impacts on natural communities must be inferred from situations where its abundance has been unintentionally modified.

The disease, and the fungus that causes it, first attracted notice in Europe around 1925 in Douglas-fir plantations established with planting stock imported from the U.S. In the 1930s, diseased Douglas-fir plantations were reported from Germany, Austria, Denmark, and the British Isles, and almost simultaneously from the northeastern U.S. Disease symptoms are chlorosis, premature loss of foliage, and reduced growth. The causal agent, *P. gaeumannii*, was found to be abundant on foliage of diseased trees and was determined to be distinct from any previously described foliage fungi from coniferous hosts (Boyce, 1940).

Following the reports of *P. gaeumannii* from diseased Douglas-fir in Europe and the eastern U.S., surveys of Douglas-fir within its native range in western North America found that *P. gaeumannii* was widespread. J.S. Boyce, the preeminent American forest pathologist of the early 20th century, wrote of *P. gaeumannii*:

> Within the natural range of Douglas-fir in western North America the fungus has been present for many years although it has passed unnoticed … because there the fungus is either not at all or so negligibly injurious to the host that it is easily overlooked. Since then the fungus has been found at such widely separated localities in British Columbia, Washington and Oregon that it must be considered generally distributed, although harmless, in the Douglas-fir region of the Pacific Coast. (1940, p. 650)

Boyce himself reported the fungus present but initially undetected on herbarium specimens he collected in Oregon and California in 1916 (Boyce, 1940). Because of the apparent widespread distribution of *P. gaeumannii* in western North America, Boyce (1940) considered the fungus to be most likely native to western North America and not of European or Asian origin. Boyce (1940) noted that while in western North America *P. gaeumannii* is a practically harmless parasite, climatic conditions more favorable to development of the fungus or increased susceptibility to disease by Douglas-fir planted as an exotic might

cause the fungus to become pathogenic and injurious to Douglas-fir planted outside its native range.

Since Boyce's (1940) characterization of *P. gaeumannii* as having a negligible effect on Douglas-fir in the Pacific Northwest, Swiss needle cast disease has become more prominent in the region. Sporadic outbreaks have been reported in Douglas-fir Christmas tree plantations since the mid-1970s (Hadfield and Douglas, 1982; Michaels and Chastagner, 1984). Starting around 1990, a decline in several Douglas-fir forest plantations in the Coast Range of Oregon began to be noticed, particularly in the vicinity of Tillamook, OR. Trees were chlorotic, had sparse crowns, and grew poorly (Hansen et al., 2000; Kastner et al., 2001). It is normal for Douglas-fir trees in the area to retain 4 to 5 years' annual complements of needles, but in severely diseased stands, trees holding more than 1 to 1 1/2 years' worth of needles were rare. In contrast to the situation described by Boyce (1940) earlier in the century, fruiting bodies of *P. gaeumannii* were conspicuous and abundant, and the symptoms of Swiss needle cast were identical to those reported from Europe. Furthermore, symptoms could be alleviated by treatment with fungicides, and *P. gaeumannii* was the only fungus that was consistently associated with disease symptoms (Hansen et al., 2000b). Since 1995, the Oregon Department of Forestry has conducted annual aerial surveys of symptomatic Douglas-fir plantations in western Oregon. The symptomatic area has grown from 53,000 ha in 1996 to more than 108,000 ha in 2003 (Oregon Department of Forestry, unpublished). Volume growth losses estimated for plantations affected by Swiss needle cast in western Oregon range from 23%, where the disease is moderate, to over 52%, where disease is severe (Maguire et al., 2002).

It has long been suspected that local climate plays a key role in the pathogenicity of *P. gaeumannii*. Boyce (1940) suggested that seasonal patterns in local climate could differentially affect fungal growth and development, and this might explain the greater virulence of *P. gaeumannii* in Europe and the eastern U.S. compared with the areas where both *P. gaeumannii* and Douglas-fir are native. Within the Douglas-fir zone of the Pacific Northwest, there is also a relationship between disease severity and local climate. Hood (1982) found higher levels of *P. gaeumannii* in southern British Columbia and western Washington in coastal forests of Vancouver Island and the Olympic Peninsula, with lower levels in the rain shadow of eastern Vancouver Island and the interior. More severe disease symptoms and greater fungal colonization are commonly observed on sites with low elevation near the coast. Recent studies have revealed a strong correlation between mean daily temperature during the winter months (December to February) and amount of fungal colonization on sites showing a range of disease severity in the Oregon Coast Range (Manter et al., unpublished), suggesting that growth rate of the fungus and consequently disease severity are strongly influenced by site microclimate.

Significant variation in *P. gaeumannii* growth and disease development has been observed over the years and across the distribution of *Pseudotsuga menziesii*, often related to subtle differences in environmental conditions and silvicultural practices. In the 1980s, increasing reports of disease came from coastal Oregon forest plantations, particularly on sites previously dominated by other tree species within the *Picea sitchensis* vegetation zone, a narrow strip of coastal forest characterized by elevations generally below 150 m, proximity to the ocean, and a moderate climate (Russell, 1981).

In a recent survey of Douglas-fir plantations growing within 29 km of the north Oregon Coast, 80% of Douglas-fir plantations surveyed occupied sites previously dominated by species other than Douglas-fir (Oregon Department of Forestry, unpublished). This suggests that due to changing forest management practices, Douglas-fir is more abundant in the coastal forests now than earlier this century. Although Douglas-fir is the natural seral dominant in the *Tsuga heterophylla* zone, which borders the *Picea sitchensis*

zone to the east, its occurrence in the *P. sitchensis* zone is more sporadic, and normally it occurs in mixtures of spruce and hemlock, not as pure stands (Franklin and Dyrness, 1973). An increase in the proportion of Douglas-fir in recent decades, its concentration in pure stands, and favorable climatic conditions probably have enabled *P. gaeumannii* to increase above historically normal levels in coastal forests, perhaps accounting for the more severe Swiss needle cast disease observed in the coastal Pacific Northwestern U.S. in recent years.

This suggests that *P. gaeumannii* may, in fact, act to regulate natural population densities of Douglas-fir in the coastal zone, where climatic conditions are more favorable to the growth of the fungus, by reducing the growth rate of Douglas-fir relative to the other species not susceptible to infection. Normally faster growing than spruce or hemlock, and widely planted because of its inherent growth advantage, Douglas-fir is not a good competitor where conditions allow *P. gaeumannii* to flourish. Where conditions favor *P. gaeumannii*, it apparently acts as a density-dependent regulator of Douglas-fir. Local land managers accept this hypothesis — they are returning to mixed-spruce hemlock forests, either by sacrificing established but poorly growing plantations and replanting with other species or by selecting against Douglas-fir in thinning if other species are present in the stand.

23.6 PATHOGENS AND OLD-GROWTH FORESTS

Many of the unique features of very old western forests, including multistoried stands, increased species diversity, large accumulations of standing dead trees and coarse woody debris, and the associated animals (e.g., cavity nesting birds), are consequences of pathogen activity. Root rots, stem decay fungi, and dwarf mistletoes all increase with stand age and often interact to shape the old-growth forest. As described above, root-rot mortality centers continue to expand through the life of the stand. As the margin of the mortality center extends, species composition within the center changes, resulting in very different tree communities inside and outside the mortality center. In many cases, disease-tolerant trees in the root-rot climax plant associations (Van der Kamp, 1991) are inherently smaller and shorter lived than the susceptible trees they replaced. Western hemlock after Douglas-fir and lodgepole pine after mountain hemlock are typical.

Stem decay fungi increase in abundance as forests age, becoming prevalent in very old forests. In most cases, young trees are as susceptible as old trees, but the chances of wound decay from falling trees or fire scars, or infection from true heart-rotting fungi such as *Phellinus pini* or *Echinodontium tinctorum*, accumulate with time, and the volume of decayed wood continually increases. The incidence of decay is often very high in old forests. In Douglas-fir stands greater than 250 years old, Boyce and Wagg (1953) found 17% of the stem volume decayed, primarily by *P. pini*, and net annual growth was negative after about age 250 years, with more volume lost to increasing decay than added through radial growth.

Pathogenic stem decay fungi also kill trees. In western forests the canker-rot fungi *Phellinus hartigii* on hemlock and *P. cancreformans* on grand fir kill the cambium and decay sapwood as well as heartwood. The heartwood decay caused by *P. pini* slowly encroaches on the functional sapwood, ultimately killing trees. The distribution of stem decay fungi in the stand is often irregular and scattered. The consequent breakage and mortality creates single-tree canopy gaps, allowing the gradual development of a second canopy layer and increasing overall species diversity.

Each of these agents exerts an increasing negative effect on net primary production of forests as they attain great age. When they act together in the same stand, the result

may be dramatic. Holah et al. (1997) briefly described an old-growth stand where laminated root rot had killed the Douglas-fir. Root-rot-tolerant western hemlock succeeded the fir. Many of the oldest hemlocks, however, had been killed by *P. hartigii* canker rot, and the remainder were severely stunted by *Arceuthobium tsugense*, hemlock dwarf mistletoe. The residual plant community was dominated by the shrub *Rhododendron macrophyllum.* In the primeval forest, such dramatically deteriorated stands must have been relatively uncommon, because stand-replacing wildfires usually recurred at intervals of several hundred years, resetting the successional clock and reducing the pathogen populations. But because many of the shrub and hardwood tree species that establish and maintain themselves in laminated root-rot mortality centers regenerate by sprouting, and because of the long survival capability of *P. weirii* in infected roots, even stand-replacing wildfire does not erase the root-rot signature.

23.7 CONCLUSIONS

The species composition and structure of forest communities are the result of the dynamics of competition and adaptation in combination with the selective effects of pathogens. The effects of pathogens on plant community structure can be subtle where native pathogen and host have evolved in close association, or conspicuous where human management activities or recently introduced invasives radically upset the equilibrium. Unlike abiotic forms of disturbance, pathogens act selectively on host species and genotypes, their effects accumulate slowly, and like their hosts, they change in response to changing opportunities. Pathogens do not have to kill their hosts to affect host fitness or the composition of communities, and their effects can be indirect. They can change the diversity of forests at both local and landscape scales, but predicting the direction of change, especially locally, toward increased or decreased diversity, or effects on a particular species, is not straightforward. The direct effects of mortality, loss of a dominant individual or group of individuals from the community, may trigger a chain of indirect effects on plant community composition as different species take advantage of newly available resources.

What regulates pathogen-induced changes in community diversity? Epidemiology and pathogenesis are obviously important. Does the pathogen spread randomly by airborne spores, directionally via insect vectors, or slowly to adjacent trees through vegetative growth? Does it kill trees outright, or reduce their reproductive or competitive fitness? Two characteristics of the forest community also seem especially important: the relative susceptibilities of the plant species present to particular pathogens and the competitive relationships of those species. Local outcomes are often site specific, strongly influenced by chance or historical events that have determined what species are present and can take advantage of the death of a competing tree.

When discussing the effects of pathogens in forest communities, it is important to distinguish nonnative pathogens from those that have evolved with their hosts. The kinds of changes are similar, and even the magnitude of the effect can be as great for an indigenous pathogen as for one introduced to a new ecosystem, but the rates of change are likely to be dramatically different. Native pathogens exist in a dynamic equilibrium with the forest community. The cycles of change may be very long, as with *P. weirii* in conifer forests, but across several tree generations we expect that populations of both pathogens and trees will stay about the same. Successful exotic pathogens, on the other hand, may drive change in plant communities beyond the limits of resiliency of the ecosystem. In the long term, a new equilibrium will be established as the pathogen is

naturalized in its new home, but the resulting community will likely be quite different from the one that existed previously.

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24

The *Epichloë* Endophytes of Grasses and the Symbiotic Continuum

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24.1 INTRODUCTION

One of the world's most intensely studied symbiotic systems is that of the grass *Lolium arundinaceum* (Schreb.) Darbysh. (= *Festuca arundinacea* Schreb.; tall fescue), with its common endophyte *Neotyphodium coenophialum* (G. Morgan-Jones et W. Gams) A.E. Glenn et al. The practical applications of this symbiosis, together with livestock toxicoses attributable to the endophyte, have fueled intense interest from mycologists, plant biologists, plant breeders, ecologists, chemists, and molecular biologists. This interest was further fueled by several remarkable facts that emerged shortly after the discovery of the endophyte (Bacon et al., 1977; reviewed in Bush et al., 1997; Malinowski and Belesky, 2000; Clay and Schardl, 2002; Panaccione and Schardl, 2003; Schardl, 2004; Schardl et al., 2004a). First, the tall fescue–*N. coenophialum* symbiotum is a systemic and long-term association in which the endophyte colonizes all aerial parts of the plant and is vertically transmitted at very high efficiency (Siegel et al., 1985). Second, the endophyte has the capability to protect the host from abiotic and biotic stresses by various means, including synthesis of anti-insect and antivertebrate alkaloids, and changes in host metabolism and architecture. Third, numerous other endophytes in cool-season grasses (subfamily Poöideae) are closely related to the tall fescue endophyte and share characteristics of systemic symbiosis, vertical transmission, bioprotection, and antiherbivore alkaloids (Figure 24.1). Fourth, although *N. coenophialum* and related endophytes are asexual, non-

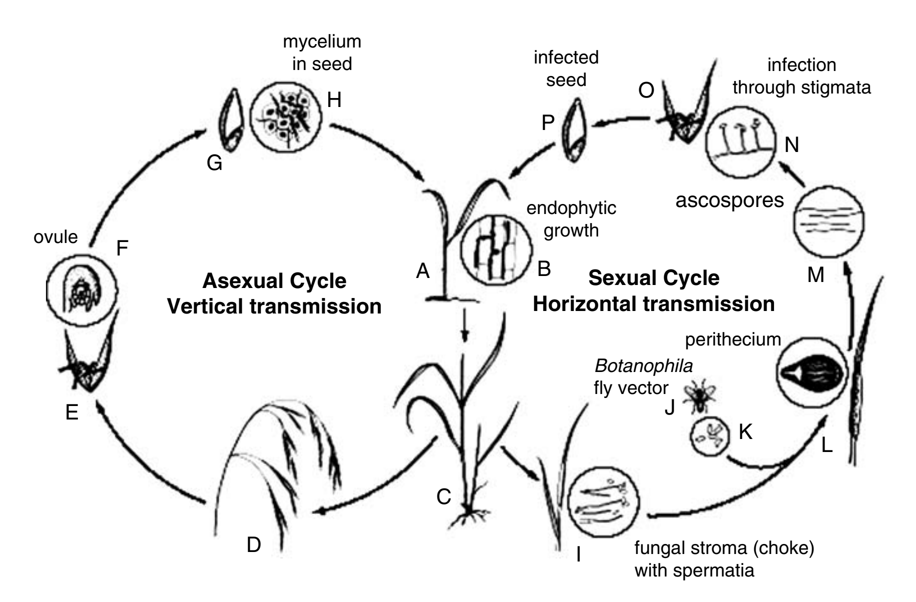


Figure 24.1 (a) Infected *Brachypodium sylvaticum* with stromata. (b) Stromata of *Epichloë sylvatica* in different stages of maturation. (c) Larval brood chambers (arrows) from which larvae emerge to feed on developing perithecia of *Epichloë typhina*. (d) Infected leaf sheath of *Lolium perenne* stained for hyphae. (e) Fungal growth (arrows) in stem and leaf meristems of *Bromus ramosus*. (f) *Neotyphodium* anamorph of *Epichloë bromicola* in culture. (g) Endophytic hyphae among aleurone cells of *Lolium perenne* seed. Magnifications: (d) ×700; (e) ×150; (f and g) ×200.

pathogenic fungi, they are evolutionary derived from sexual plant pathogens, namely, *Epichloë* spp.

Seed-transmitted grass endophytes have been known for well over a century (Freeman, 1904), but only since 1982 have the characteristics and relationships of these endophytes been sufficient to begin describing new *Neotyphodium* spp. (Morgan-Jones and Gams, 1982). Also, development of the means to culture most endophytes and to introduce them into endophyte-cured plants of their native hosts (Latch and Christensen, 1985) or other grasses (Koga et al., 1993; Christensen et al., 2000; Johnson-Cicalese et al., 2000) has led to a better understanding of niche specialization of the endophytes. Furthermore, prior to 1993, all *Epichloë* spp. known from poöid grasses were classified as *Epichloë typhina*, but since phylogenetic analysis began to be applied to the problem, the characterization of new *Epichloë* spp. has gone hand in hand with descriptions of numerous new *Neotyphodium* spp.

An understanding of the ecological and evolutionary implications of the epichloë endophytes requires recognition of how the diversity of symbiotic types relates to taxonomic diversity of the partners involved. All of these symbioses involve fungal endophytes systemically inhabiting host grasses. In many the symbioses are maintained through host generations (inherited), and in many the symbiont is capable of horizontal transmission (Figure 24.2). But the latter process is promoted by the sexual cycle, which entails symbiosis with fly species of the genus *Botanophila* (class Diptera, order Anthomyiidae) (Figure 24.1C). Molecular data such as isozymes (Leuchtmann and Clay, 1990), microsatellites (Moon et al., 1999), and DNA sequences have indicated unexpected complexities underlying evolutionary relationships between *Epichloë* species (Craven et al., 2001b)

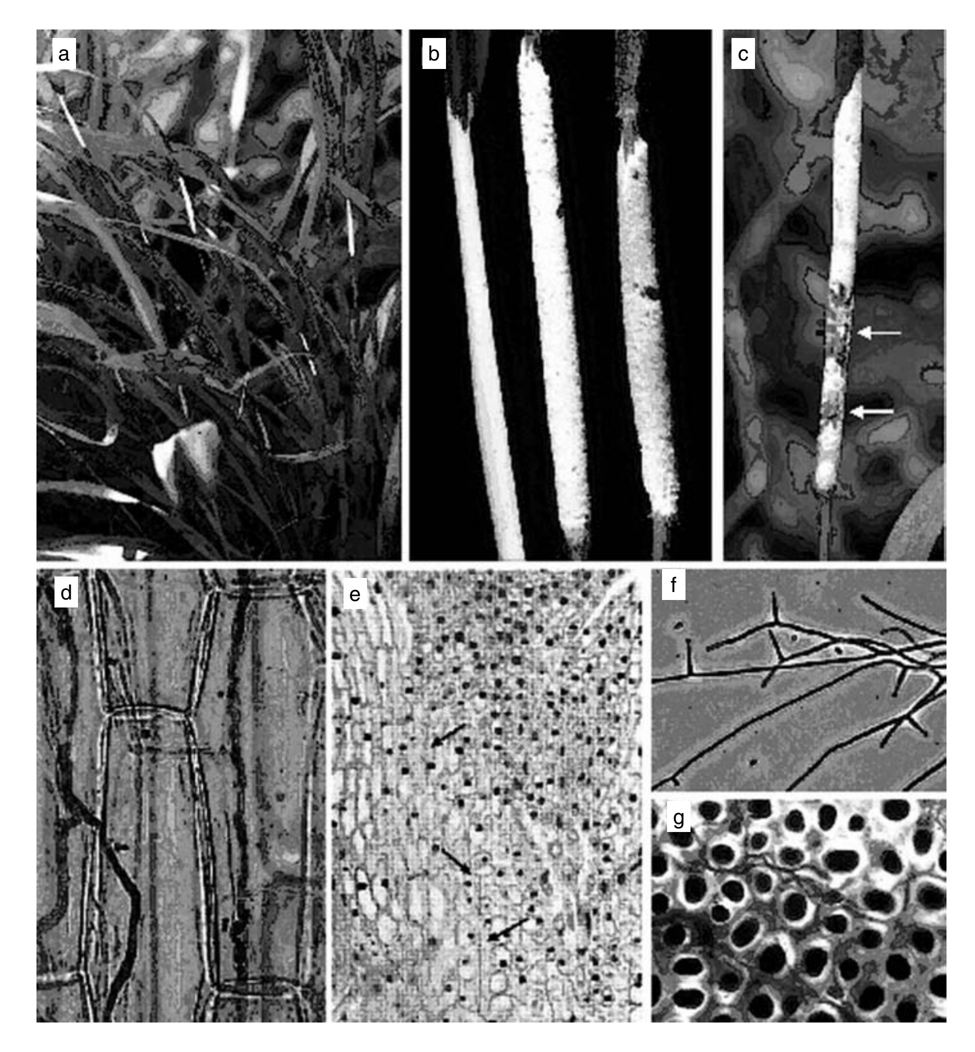


Figure 24.2 **(See color insert following p. 460.)** Coordinated life cycles of *Epichloë* spp. and their grass hosts. Pleiotropic symbionts undergo both life cycles on different tillers, whereas other *Epichloë* species may not permit seed set and are thus obligately sexual and horizontally transmitted as indicated at right. The asexual species (*Neotyphodium* spp.) undergo only the vertical transmission cycle indicated at left.

and the evolution of *Neotyphodium* species from *Epichloë* species (Moon et al., 2004) (Figure 24.3).

In this review we discuss each of the *Epichloë* and *Neotyphodium* spp. described to date, in reference to its discovery, history, ecology, biochemistry, and evolution.

24.2 CHARACTERISTICS OF *EPICHLOË* SPECIES

24.2.1 *Epichloë typhina* (Pers.) Tul.

Prior to 1993, all members of the genus *Epichloë* found in association with the grass subfamily Poöideae were classified in the single species *E. typhina*, based on the characteristic (pre)sexual stroma, which surrounds the sheath of the flag leaf on a generative

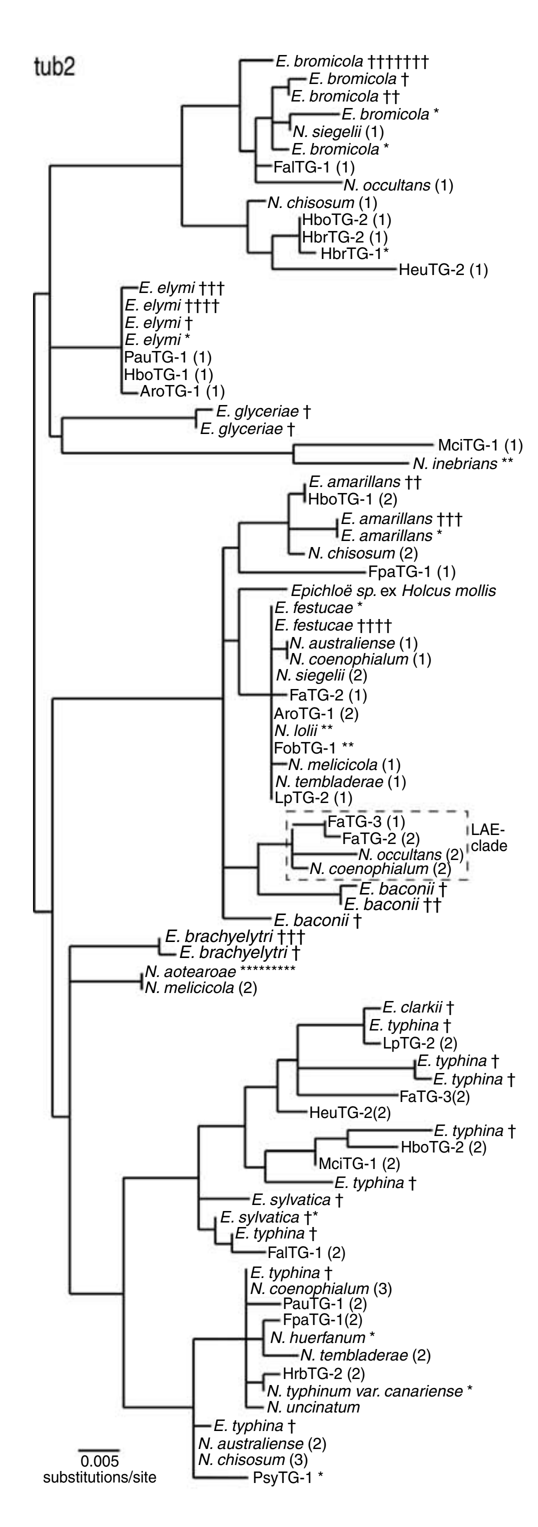


Figure 24.3 Phylogenetic analysis of introns in the beta-tubulin gene (*tub2*) of *Epichloë* and *Neotyphodium* spp. Nonhybrid endophytes are designated with daggers (†) if isolated from stroma-bearing symbiota and with asterisks (*) if from asymptomatic symbiota. (The number of symbols after each taxon indicates the number of isolates with that sequence.) For hybrid endophytes, the distinct alleles are indicated by numbers following the species or taxon designation. This phylogram was inferred by maximum likelihood and should be considered unrooted, although the tree is drawn with the midpoint root placed at the left edge.

tiller of the host plant (Figure 24.1A and B). The resulting choke disease is characterized by an inability of the tiller to further develop and set seeds. In 1993, White (1993) described two new *Epichloë* species based on morphological differences of the ascospores, host specificities, and mating (in)compatibilities. In this study, he also selected as type for *E. typhina* stromata on *Dactylis glomerata* L. that had been collected by Persoon. As other host–*Epichloë* spp. associations were identified, many were considered referable to *E. typhina*, primarily based on their mating compatibilities with *E. typhina* on *D. glomerata* (Leuchtmann and Schardl, 1998). Furthermore, the consistent observation was that each host characteristically harbored only a single sexual *Epichloë* species. (Occasionally, a host might also harbor an asexual relative, namely, *Neotyphodium* species, as will be discussed later.) So far, strains interfertile with *E. typhina*, and referable to that species, have been identified in *D. glomerata*, *Anthoxanthum odoratum* L., *Brachypodium pinnatum* (L.) P. Beauv., *Brachypodium phoenicoides* (L.) Roem. & Schult., *Lolium perenne* L., *Phleum pratense* L., *Poa nemoralis* L., *Poa pratensis* L., *Poa trivialis* L., and *Puccinellia distans* (L.) Parl. (Leuchtmann and Schardl, 1998; A. Leuchtmann, personal observations). It is very likely that more hosts will continue to be identified.

Epichloë typhina has not been found to transmit vertically in seeds of any of its known hosts except *Poa nemoralis* (Leuchtmann and Schardl, 1998; A. Leuchtmann, personal observations). In all of the other associations, and in some associations with *Poa nemoralis*, the infected plants rarely produce any seeds. This is because stromata emerge on all or nearly all of the generative tillers and the encased inflorescences fail to emerge from the surrounding leaf sheath, thus failing to mature. However, in extensive observations of *E. typhina*-infected *L. perenne*, some emergent inflorescences have been observed (particularly in greenhouse-cultivated plants), yet seed production by these inflorescences was very low. The occasional seeds obtained from such inflorescences were germinated, and the progeny plants were tested for *E. typhina*, which was absent from those plants (Chung and Schardl, 1997a). Curiously, the inflorescences had not escaped infection, and hyphae of characteristic *E. typhina* morphology were abundant in association with the stigmata, though little or none could be observed in the ovaries. A possibility, as yet untested, is that the presence of abundant *E. typhina* hyphae prevented seed set (perhaps by interfering with fertilization), and those few seeds that were obtained were from florets that had escaped infection.

Despite the observations that *E. typhina* is not transmitted vertically via seeds in *L. perenne*, *P. trivialis*, or *D. glomerata* (Chung and Schardl, 1997a; A. Leuchtmann, personal observations), the fungus is nevertheless seed transmissible because ascospores produced on stromata are ejected into the atmosphere and can infect the florets of neighboring plants. This results in seeds that bear the fungus and that when germinated, give rise to systemically infected plants. This horizontal seed transmissibility of *E. typhina* has been demonstrated in the host plant, *L. perenne*, with ascospores generated from a mating of mat-1 (mating type 1) stromata on *L. perenne* with mat-2 isolates from *D. glomerata* (Chung and Schardl, 1997a). It seems to us very likely that the recent epidemic of *E. typhina* on *D. glomerata* in Oregon arose from import of infected seeds from Europe, although others have suggested the pathogen is emerging due to climate change (Pfender and Alderman, 1999).

How is it that this fungus is capable of horizontal infection of developing seeds, but not vertical transmission? Currently there is no evidence to bear on this question. We speculate that the presence of *E. typhina* hyphae in abundance early in floret development triggers in the host a defense that prevents invasion of the ovule. If horizontal transmission is similar to that of the related ergot fungi — *Claviceps* spp. (Tudzynski et al., 1995) — the route would be via the exerted stigma. Thus, exposure to the hyphae may occur later,

and there might also be less abundant hyphae in the infected floret during horizontal transmission, compared with the case for vertical transmission of other endophytes and of *E. typhina* in *P. nemoralis*. This possible explanation leads to a prediction that in associations characterized by vertical transmission there will be more control of fungal proliferation in the florets of asymptomatic inflorescences. Curiously, whereas in some *P. nemoralis–E. typhina* symbiota all generative tillers exhibit stromata, in others most or all of the inflorescences are normal and produce normal seeds bearing the endophyte (A.L., personal observations).

The phylogenetic relationships of *E. typhina* with other *Epichloë* species are unusual in that *E. typhina* appears to be paraphyletic to *E. sylvatica* Leuchtm. et Schardl and *E. clarkii* J.F. White (Craven et al., 2001b) (Figure 24.3). Also at odds with the phylogenetic relationships are the interfertility relationships: *E. typhina* and *E. clarkii* are interfertile (and classified as mating population MP-I), but are not interfertile with *E. sylvatica* (classified as MP-VII) (Leuchtmann and Schardl, 1998). Two possible and nonexclusive explanations are that speciation within this group (collectively termed the *E. typhina* complex (ETC)) does not follow the often assumed cladistic pattern, or that *E. typhina* as currently circumscribed represents several cryptic species.

A quantitative Southern blot analysis of the beta-tubulin gene (*tub2*) indicated a genome size estimate of 28.8 ± 3.5 Mb for *E. typhina* ATCC 200736 (Kuldau et al., 1999). Electrophoretic karyotype analysis indicated five chromosomes, with their summed sizes giving a 32.5-Mb estimated genome size (Kuldau et al., 1999). The quantitative Southern blot is likely to be the more accurate estimate.

24.2.2 *Epichloë clarkii* J.F. White

This species was, along with *E. baconii*, one of the first new *Epichloë* species described in the 1990s (White, 1993), and *E. clarkii* appears to be restricted to the host *Holcus lanatus* L. Ascospore morphologies of *E. clarkii* and *E. typhina* differ in that the former disarticulates into two spear-shaped part-spores before or upon ejecting, whereas the latter ejects whole filamentous spores, eight spores from each ascus. In mating tests in sympatric populations located in Yorkshire, Britain, *E. clarkii* appeared incapable of mating with *E. typhina* (White, 1993). However, among the isolates of *E. clarkii* and *E. typhina* in Switzerland, there is an almost unrestricted interfertility observed in mating experiments (Leuchtmann and Schardl, 1998). Nevertheless, there is no evidence of natural productive interbreeding of these species. All six isolates of choke agents so far obtained from *H. lanatus* share identical sequences of the first three introns in *tub2*, and all isolates of *E. typhina* from other hosts have at least one and usually many nucleotide substitution differences from *E. clarkii*. Similar results have been obtained from analysis of introns in the gene for translation elongation factor 1-alpha (*tef1*) (Craven et al., 2001b). Thus, to date there appear to be no haplotypes (alleles) shared between *E. clarkii* and *E. typhina*.

A possible reason for genetic isolation of *E. clarkii* from *E. typhina* is specificity of the anthomyiid flies responsible for cross-spermatizing stromata (Figure 24.1C). Bultman and Leuchtmann (2003) have observed that *Botanophila* spp. flies predominantly carry spermatia from a single host, indicating that there is some specialization of flies in their visitation behavior. Only in two instances were spermatia of *E. clarkii* mixed with those of *E. typhina* in the fly feces. Nevertheless, in experimental plots where both hosts were present and allowed to cross-fertilize via the fly vector, successful mating obviously did not occur between *E. typhina* on *D. glomerata* and *E. clarkii* on *H. lanatus.* Should interspecific mating occur in rare instances, species could still remain reproductively isolated if postzygotic isolation mechanisms are operating. Hybrid ascospores could have

reduced ecological fitness compared with those resulting from intraspecific matings, for example, through impaired capabilities to disperse, germinate, or colonize new host plants. Host specificity is a very common feature of *Epichloë* species, and even among *E. typhina* isolates there are heritable differences in host preference (Chung et al., 1997). Such host preferences may synergize with specificity of *Botanophila* interactions in maintaining reproductive isolation and promoting speciation of the fungal symbionts.

24.2.3 *Epichloë sylvatica* Leuchtm. et Schardl

This species has been described based on its sexual incompatibility with other known *Epichloë* species, its association with its only known host, *Brachypodium sylvaticum* (Huds.) Beauv., and some distinct morphological features of its perithecia and ascospores (Leuchtmann and Schardl, 1998) (Figure 24.1A and B). The species is extremely widespread and appears to be obligately associated with *Bp. sylvaticum* plants throughout Europe and Asia, with a nearly 100% infection rate. However, in most populations infected plants do not display stromata; rather, an asexual, seed-transmitted form prevails. Mating tests indicate interfertility between isolates that form stromata on *Bp. sylvaticum*, and between some but not all seed-transmissible isolates from this host. Fertile *E. sylvatica* isolates are classified as MP-VII (Schardl et al., 1997).

Nonstromatal forms of *E. sylvatica* and sexual strains may co-occur as two distinct subpopulations on the same host grass and therefore do not appear to be panmictic (Bucheli and Leuchtmann, 1996), probably because the difference in their life cycles prevents outcrossing. In many locales the asexual, seed-transmitted form is the only one present, often with single clones being widespread. In contrast, stroma-forming strains are rare and genetically more diverse. Plants with mixed symptoms, previously considered to be the result of a balanced host interaction, are often simultaneously infected by different genotypes, one sexual and the other asexual (Meijer and Leuchtmann, 1999). Sexual strains, however, may also be capable of seed transmission when special circumstances allow for flowering of infected culms. This distinguishes them from many strains of the closely related *E. typhina*, but is similar to the situation in *P. nemoralis–E. typhina* symbiota.

Contagious spread of sexual *E. sylvatica* occurs not only by invasion of ovules and seeds after infection of grass florets, but also by direct infection of vegetative tillers. In field experiments, up to 34% of uninfected plants of *Bp. sylvaticum* transplanted to sites where stromata of *E. sylvatica* were present became infected after 2 years, presumably by ascospores invading the meristematic zone of tiller buds (Brem and Leuchtmann, 1999). Horizontal transmissions in unmanipulated stands may actually be less frequent because most plants are already infected (usually with asexual *E. sylvatica*), so that any newly arrived strain in a plant must compete with the endophyte already resident in that plant. In fact, recent findings suggest that infection by seed-transmitted endophytes causes host plants to become less susceptible to infection by choke-forming strains (Meijer and Leuchtmann, 2001).

The high incidence of infection by asexual strains found in *Bp. sylvaticum* populations suggests that infected plants may have a selective advantage over uninfected plants. Among the possible mechanisms providing benefits to this association is increased resistance to herbivory. In laboratory experiments, the insect herbivore *Spodoptera frugiperda* performed significantly better on a diet of uninfected leaves of *Bp. sylvaticum* than the infected control, and in natural populations of the host, unknown microherbivores caused considerably more damage to stroma-bearing tillers than to those that were asymptomatically infected (Brem and Leuchtmann, 2001). Thus, asexual strains of *E. sylvatica* appear to be better able to protect their hosts against insect herbivory than are the sexual strains. Stimulatory effects on growth or improved competitive abilities, which are well docu-

mented for grasses infected by other endophytes, have not been found for *Bp. sylvaticum* (Brem and Leuchtmann, 2002). An intriguing idea for explaining the widespread occurrence of asexual infections is that seed-transmitted *E. sylvatica* could alter the reproductive system of the host grass, such that seed production and hence endophyte transmission is increased. *Bp. sylvaticum* is the only selfing species of the genus — which allows for dependable and much higher production of fertile seed compared with the outcrossing species (Schippmann, 1991) — and is the only *Brachypodium* species known to be infected by a vertically transmitted endophyte. However, whether the evolution of self-compatibility in *Bp. sylvaticum* was promoted by endophyte symbiosis or existed before the endophyte evolved and facilitated the success of this association is not known and needs to be investigated.

24.2.4 *Epichloë baconii* J.F. White

This species, described in 1993 from material identified in Yorkshire, Britain (White, 1993), has been found in several *Agrostis* species and *Calamagrostis villosa* (Chaix) J.F. Gmel. In addition to these host associations, *E. baconii* is morphologically characterized by distinct ascospores disarticulating into cylindrical, one-septate part-spores. In associations with *Agrostis* and *Calamagrostis* spp., all or nearly all generative tillers of the host are choked and possess *E. baconii* stromata. There have been no indications that *E. baconii* can be vertically transmitted in seeds. However, the infections are systemic, and new plant tillers, as well as stolons, carry these infections. This species is equivalent to mating population MP-V. However, it is possible that *E. baconii* represents two (or more) cryptic species. Isolates from *Agrostis* spp. group in a clade but are genetically distant from the sole isolate from *C. villosa* analyzed to date (Craven et al., 2001b). As with *E. typhina*, the possibility of cryptic species encompassed by the current circumscription of *E. baconii* remains to be investigated.

As a single interfertility group, MP-V is more diverse than any other except *E. typhina* (Leuchtmann and Schardl, 1998). Furthermore, a very limited interfertility with *E. festucae* has been observed, as described below.

24.2.5 *Epichloë festucae* Leuchtm. et al.

In 1933, Kathleen Sampson (1933) noted a fundamental difference between the symbiotic interactions of *D. glomerata* and *Festuca rubra* L. with *E. typhina* (as circumscribed at the time). Whereas in *D. glomerata* all tillers were choked, in *F. rubra* many of the tillers gave rise to seeds bearing the fungus. Sampson suggested that the fungus inhabiting *F. rubra* might be a distinct species. In 1994, the species *E. festucae* was described based on morphological and phylogenetic relationships among choke agents commonly found in *F. rubra* and other *Festuca* species (Leuchtmann et al., 1994). *E. festucae* now represents a model for pleiotropic (mixed-strategy) symbiosis, whereby both antagonistic effects (choke disease) and mutualistic effects — namely, protection of the plants and seeds from herbivory and other stresses — occur simultaneously between host and symbiont individuals (Schardl, 2001).

Since its description, *E. festucae* has also been identified in *Lolium giganteum* (L.) Darbysh. (= *Festuca gigantea* L.), *Koeleria cristata* Pers., *Festuca pulchella* Schrad. (Leuchtmann et al., 1994), *L. pratensis* (Huds.) Darbysh. (U. Hesse and C.L.S., personal observation), and possibly *L. arundinaceum* (Triebel, 1996). Isolates from *L. giganteum* were interfertile with the stromata produced on naturally infected *F. rubra* plants (Wilkinson et al., 2000), but an isolate from *K. cristata* was not (Craven et al., 2001b). Sequence relationships also identified close relatives in several grasses where choke was not observed. In some cases, these related strains were capable of acting as males in productive

matings to *E. festucae* stromata (conidia from cultures were used as spermatia), but in other instances, they failed to cause productive mating. Isolates of *Neotyphodium lolii*, a common endophyte of *L. perenne*, were indistinguishable from *E. festucae* by DNA sequence comparisons, but did not mate with *E. festucae* stromata (Moon et al., 2004). When another endophyte from *L. perenne* was used as a male, abundant ascospores were obtained, but the ascospores were not ejected and appeared to be nonviable (Moon et al., 2000). Currently, classification as *E. festucae* is based primarily on DNA sequence relationships, with the added criteria of ascoma and ascospore morphologies, and the ability to act as a male in matings with *E. festucae* tester strains to generate abundant ascospore-bearing ascomata (whether or not those ascospores are viable). The mating population MP-II encompasses *E. festucae* on *F. rubra*, together with *E. festucae* strains with which they are interfertile.

The only successful interspecific mating to date has been between *E. festucae* and an *E. baconii* isolate from *Calamagrostis villosa* (Chaix) J.F. Gmel. (Leuchtmann and Schardl, 1998). Though ascospores were obtained, they were not ejected, and the vast majority appeared to be nonviable. However, two viable ascospores were rescued from a fertilized stroma and subjected to Mendelian analysis for allozymes. Segregation of alleles was as expected for matings of two haploid fungi, demonstrating that the ascospores represented true interspecific hybrids.

E. festucae is unique in that it is the only species known to produce all four classes of endophyte-associated alkaloids, although no single isolate is known to produce all of these alkaloids. Genetic polymorphisms associated with alkaloid profiles provide a potential means to identify and clone the genes for alkaloid biosynthesis. For example, genetic analysis indicated a single locus associated with the capability to produce lolines *in symbio*, and that locus was linked to a DNA polymorphic marker (Wilkinson et al., 2000). That marker was identified as a portion of a likely biosynthetic gene, which is related to fungal homocysteine synthase genes, has homologs only in known loline alkaloid-producing species, and whose homolog in *Neotyphodium uncinatum* W. Gams et al. is highly expressed in cultures during loline alkaloid production but not in nonproducing cultures.

A quantitative Southern blot analysis of the *tub2* gene indicated a genome size estimate of 28.5 ± 3.5 Mb for *E. festucae* ATCC 90661 (Kuldau et al., 1999). Electrophoretic karyotype analysis indicated five chromosomes, with their summed sizes giving a 35-Mb estimated genome size (Kuldau et al., 1999). The quantitative Southern blot is likely to provide the more accurate genome size estimate for this isolate. A similar karyotypic analysis of other isolates of *E. festucae* suggested that some may have six chromosomes (G.A. Kuldau and C.L. Schardl, unpublished data).

24.2.6 *Epichloë amarillans* J.F. White

Another pleiotropic symbiosis is between *Agrostis* spp. and *E. amarillans* (White, 1994). In these associations some of the tillers are choked, but most bear seeds in which the fungus is disseminated. This symbiont is also found in *Sphenopholis* spp. and probably in *Calamagrostis canadensis* (Michx.) P. Beauv. As pleiotropic symbioses, both vertical and horizontal transmission may occur, though some infected plants are not observed to produce any asymptomatic tillers on which seeds can be produced (Clay and Leuchtmann, 1989; White, 1994; Schardl and Leuchtmann, 1999). Although *E. amarillans* infects members of the same grass genera as *E. baconii*, the former is a North American species and the latter is European. Also, *E. amarillans* and *E. baconii* are related to each other and to *E. festucae*, but so far *E. amarillans* has not exhibited even a low level of interfertility with these related *Epichloë* spp. Isolates of *E. amarillans* from *Agrostis* spp. and *Sphenopholis* spp. are interfertile and classified as MP-IV.

An asymptomatic *Elymus virginicus* L. plant in Red River Gorge near Slade, KY, was found to bear an *E. amarillans* isolate (Moon et al., 2004). This isolate is precluded from mating in nature, due to the absence of stroma expression (hence producing neither spermatia nor female hyphae). But mating tests can be conducted using the cultured fungus as a source of conidia, which serve as spermatia. In this way, it has been demonstrated that the isolate is capable of mating with *E. amarillans* stromata on *Agrostis perennans* (Walter) Tuck. (Moon et al., 2004). It is of interest that the asymptomatic symbiosis was on a host plant in a genus (*Elymus*) unrelated at the tribe level to those on which *E. amarillans* is known to produce stromata. Similar situations were also observed for isolates of *Epichloë elymi* Schardl et Leuchtm. and *Epichloë bromicola* Leuchtm. et Schardl, as described later in this review. Such observations suggest that *Epichloë* species occasionally colonize new hosts to generate asymptomatic associations in which the fungus is incapable of producing its sporogenous stage leading to horizontal transmission. Should such a situation arise for most plant-associated fungi it would be unsustainable, but because of the seedborne nature of many *Epichloë* species, the result can be a mutualistic association that is inherited by subsequent host generations.

24.2.7 *Epichloë elymi* Schardl et Leuchtm.

This is also a pleiotropic symbiont, recently described from North American grasses (Schardl and Leuchtmann, 1999). Stromata of *E. elymi* have so far been observed only in association with *Elymus virginicus*, *Elymus villosus* Muhl. ex Willd., *Elymus canadensis* L., and *Elymus hystrix* L. (= *Hystrix patula* Moench), all native to North America. In addition, an asymptomatic plant of *Bromus kalmii* A. Gray (= *B. purgans* L.) contained an isolate that was interfertile with *E. elymi* when its conidia were used as spermatia (Moon et al., 2004). The interfertility group corresponding to *E. elymi* is MP-III. Seeds of *Elymus* spp. plants that carry *E. elymi* invariably also bear this symbiont. Thus, *E. elymi* shares the characteristic of *E. festucae*, *E. brachyelytri* Schardl et Leuchtm., *E. amarillans*, *E. sylvatica*, and some *E. typhina* in being seed transmissible. Experiments with the *E. elymi–El. virginicus–Botanophila* sp. system have established that *E. elymi* is heterothallic (Bultman and White, 1988) and that the *Botanophila* sp. fly is a mutualist and principal vector of *E. elymi* spermatia (Bultman et al., 1995, 1998). These observations appear to extend to all *Epichloë* species on poöid grasses, which rely on *Botanophila* spp. flies for their spermatization, and in turn provide habitat and food to the fly larvae (Kohlmeyer, 1956; Kohlmeyer and Kohlmeyer, 1974).

24.2.8 *Epichloë brachyelytri* Schardl et Leuchtm.

This species (Schardl and Leuchtmann, 1999) is a pleiotropic symbiont of *Brachyelytrum erectum*, a C_3 North American grass with an interesting phylogenetic relationship to other grasses. Morphological characterization of *Brachyelytrum* led to its classification by different taxonomists in different grass subfamilies. Some placed the genus in the Poöideae, and some in the Bambusoideae. In molecular phylogenetic studies, *Be. erectum* usually occupies the position most basal to the Poöideae (Davis and Soreng, 1993; Catalán et al., 1997). If this grass and its associated tribe (Brachyelytreae) is considered a member of the Poöideae, then all of the *Epichloë* species recognized by modern taxonomists (that is, with characteristics of the originally described *E. typhina*) are symbionts of poöid grasses.

Plant–*E. brachyelytri* symbiota in the wild can sometimes be completely asymptomatic. Even when stromata are produced, these often remain unvisited by *Botanophila* flies and, hence, unmated. The first documented case of mated *E. brachyelytri* stromata ejecting viable ascospores was in Acidalia, Sullivan County, NY, in a very small *E. brachyelytri* population. This discovery led to the description of the species (Schardl and

Leuchtmann, 1999). Isozyme relationships (Leuchtmann and Clay, 1993) and, later, DNA sequence relationships (Craven et al., 2001b) indicated that isolates from asymptomatic *Be. erectum* plants and from unfertilized stromata on this host were also referable to *E. brachyelytri*. Therefore, to date the only endophyte known from *Be. erectum* is *E. brachyelytri*, which in turn has been associated only with that host. However, strains of *E. elymi* from *Elymus* spp. do form stable associations with *Be. erectum* when artificially introduced to seedlings of the grass (Leuchtmann and Clay, 1993), indicating that there is potential susceptibility to more than one endophyte.

The host has proven difficult to cultivate, so *E. brachyelytri* tester strains have not been established for mating tests. It is assumed that isolates from this host showing close DNA sequence relationships are members of a single interfertility group, tentatively designated MP-IX (Schardl et al., 1997).

24.2.9 *Epichloë glyceriae* Schardl et Leuchtm.

So far, this species has been found only in *Glyceria striata*, though early surveys of *E. typhina* s.l. (Farr et al., 1989) suggest other *Glyceria* species might also be hosts. The species corresponds to interfertility group MP-VIII. In contrast to the other three species described from North America, *E. glyceriae* almost completely eliminates seed production by infected plants (Schardl and Leuchtmann, 1999). Because seeds are produced so rarely on these plants, information is not yet available as to whether the fungus is vertically transmissible. Although the suppression of seed set is a clear antagonistic effect of this endophyte, infected plants nevertheless may also gain substantial benefits from the symbiosis. For example, in a survey of endophyte-associated effects on insects, *E. glyceriae*-infected *G. striata* exhibited the most potent activity against fall armyworm (Cheplick and Clay, 1988). It appears that the endophyte causes almost complete lethality to this herbivorous insect.

Recent studies (Pan and Clay, 2002, 2003) indicate that *E. glyceriae* alters host allocation of biomass during vegetative growth and clonal proliferation. Specifically, plants symbiotic with *E. glyceriae* allocate greater biomass to stolons, whereas plants without *E. glyceriae* allocate more biomass to vegetative tillers. These results are largely consistent whether comparing naturally infected and uninfected plants (Pan and Clay, 2002) or naturally infected plants with clones of those plants from which the endophyte had been eliminated by fungicide treatment (Pan and Clay, 2003). In the latter study, a particularly dramatic effect was on stolon numbers, with most *G. striata* genotypes exhibiting greater primary and secondary stolon numbers when infected with *E. glyceriae*. Stolons may enhance the exploration of multiple microenvironments, as well as provide an efficient means of daughter ramet production (Pan and Clay, 2003). Considering that seeds can provide similar functions, increased stolon production might at least partially compensate for the loss of seed production due to the *E. glyceriae* infection.

24.2.10 *Epichloë bromicola* Leuchtm. et Schardl

This species is predominantly associated with European species of genus *Bromus*. It is found on *Bromus erectus* Huds., a species common in dry calcareous grassland, and on two woodland grass species, *Bromus benekenii* (Lange) Trimen and *Bromus ramosus* Huds. (Leuchtmann and Schardl, 1998). Recently, it has also been identified among isolates from *Hordelymus europaeus* (L.) Harz (Moon et al., 2004; A.L., personal observation). Infection frequency is usually more than 80% in natural populations of *B. benekenii* and *B. ramosus* (Leuchtmann, 1996), whereas infection of *B. erectus* is more scattered and only locally abundant. The different host strains are interfertile among each other experimentally and thus represent a single biological species (MP-VI) that is distinct from all other *Epichloë*

species. However, strains differ in their life cycles. Those naturally infecting *B. erectus* are always choke forming and fail to transmit in host seed, whereas strains from *B. benekenii*, *B. ramosus*, and *He. europaeus* lack a sexual stage and are purely seed transmitted (Figure 24.1g). Moreover, analysis of amplified fragment length polymorphisms (AFLP) polymorphisms and intron sequences of *tub2* and *tef1* genes indicate genetically distinct clades of strains from the three *Bromus* spp., suggesting different host-adapted races of *E. bromicola* (Brem and Leuchtmann, 2003). In reciprocal inoculation tests with plant seedlings, seed-transmitted isolates from *B. benekenii* and *B. ramosus* could easily be moved among the asymptomatic hosts, but not to *B. erectus*, indicating that they are host specific (Brem, 2001). In contrast, the stroma forming *E. bromicola* strains were broadly compatible with all three hosts. The seed-transmitted strains infecting woodland grasses may represent incipient species that have not yet completely developed incompatibility barriers to mating but are *de facto* reproductively isolated through loss of sexuality and adaptation to different hosts.

It is assumed that asexual strains arose from within sexual populations on *B. erectus* after host shifts to *B. benekenii* and *B. ramosus* (Brem and Leuchtmann, 2003). The emergence of the nonstromatal populations of *E. bromicola* apparently involved a change of the transmission mode from purely horizontal in sexual strains to purely vertical in asymptomatic strains. Interestingly, there is no intermediate stage with a balanced type of symbiosis known in *E. bromicola*. Thus, adaptational changes on the new hosts may have included both loss of sexuality and the development of the mechanism to grow from flowering meristems into ovules and seeds. If seed transmission were an ancestral trait, reversal to that mode of transmission may have been simple, but if not, mutual adaptations in the compatibility and relative growth speed of host and fungus within tissues were necessary. Selective forces that promoted seed transmission may have been the increased mutualistic effects after restoring host fertility and the more efficient dissemination of the endophyte within seeds. Indeed, infected *B. benekenii* plants have superior competitive abilities compared with uninfected plants (Brem and Leuchtmann, 2002) and may be better protected from insect herbivores (Biber and Leuchtmann, unpublished data). Moreover, the very high rates of infection in natural populations emphasize the increased success of seed-transmitted associations of *E. bromicola*.

24.3 CHARACTERISTICS OF *NEOTYPHODIUM* SPECIES

24.3.1 *Neotyphodium typhinum* (G. Morgan-Jones et W. Gams) A.E. Glenn et al.

Prior to 1982 the anamorph of *E. typhina* (then broadly defined) was classified as *Sphacelia typhina* Tul. A reclassification in 1982 (Morgan-Jones and Gams, 1982) placed it into *Acremonium* Link and into the new section *Albo-lanosa* G. Morgan-Jones et W. Gams. Subsequent reclassification of all *Albo-lanosa* as *Neotyphodium* spp. (Glenn et al., 1996) gave the binomial, *N. typhinum*. As defined, *N. typhinum* has typical anamorph characteristics of all *Epichloë* species associated with poöid grasses, so *N. typhinum* or *A. typhinum* cannot be considered to apply only to the anamorph of *E. typhina sensu stricto*. For example, *A. typhinum* var. *bulliforme* White (White, 1992) is an anamorph of *E. baconii*. Essentially, *N. typhinum* possesses culture characteristics of white, usually cottony aerial mycelium with septate, unpigmented vegetative hyphae; perpendicular branching of single, discrete conidiogenous cells; phialidic conidiation; and hyaline, one-celled conidia that are small (ca. 4×2.5 μm), asymmetrical, and navicular to nearly ellipsoid (Figure 24.1F). Because this taxon can represent the anamorph of *E. typhina* and other *Epichloë* spp., it

cannot be assumed to be asexual or capable of vertical transmission. This is in contrast to the other *Neotyphodium* taxa discussed herein, all of which are asexual and depend on vertical seed transmissibility for their reproduction.

24.3.2 *Neotyphodium coenophialum* (G. Morgan-Jones et W. Gams) A.E. Glenn et al.

This is the first species of truly asexual, vertically transmissible clavicipitaceous endophyte to be described. It is very likely that it was discovered by Neill in the early 20th century (Neill, 1941), but was not widely known until its rediscovery in 1977 by E.S. Luttrell's group (Bacon et al., 1977). Events leading up to its rediscovery center around the development and rapid widespread use of its host, tall fescue (*Lolium arundinaceum* = *Festuca arundinacea*), as a new forage grass in the U.S.

In the mid-20th century, tall fescue was the target of intense inquiry that began with the dust bowl and with an encounter between agronomy professors from the University of Kentucky and a Menifee County farmer (Buckner et al., 1979; Stuedemann and Hoveland, 1988). It is important to recall that the Great Depression of the 1930s was aggravated in the U.S. by recurrent severe droughts during which topsoil blew away in huge dust storms (hence, the dust bowl). In 1931, the professors were attending a local field day, after which William O. Suiter invited them to his hillside farm. There he showed the professors a stand of pasture grass that was particularly persistent even during the drought. (Reputedly, some time prior to 1887 this grass was brought over the Atlantic in the hay used to pack a shipment of English bone china.) Prof. E.N. Fergus was impressed with this grass and developed it into tall fescue cultivar Kentucky 31, which was then aggressively promoted by Extension professors for pastures and by the U.S. Soil Conservation Service to help control erosion. However, by the 1950s a rancorous controversy, later to be known as the fescue war, emerged within the University of Kentucky Agronomy Department. This controversy centered on a dispute as to whether animals that grazed tall fescue performed as well as expected. By the mid-1970s the problem of fescue toxicosis in cattle and other livestock was well recognized, and the symptoms were regarded as generally similar to ergot poisoning caused by *Claviceps purpurea* (Fr.) Tul. (Raisbeck et al., 1991). Thus, Bacon et al. (1975) investigated the occurrence in pastures of *Balansia* species, systemic grass parasites related to ergot fungi. These studies led to a rediscovery of the tall fescue endophyte now known as *N. coenophialum*, and the association of this endophyte with ergot alkaloids in tall fescue (Bacon et al., 1977). Of particular note was ergovaline, an ergopeptine similar but not identical to toxins in *Cl. purpurea*-contaminated flour (Lyons et al., 1986). A great abundance of experimental evidence indicates that *N. coenophialum* causes the toxicoses associated with tall fescue (Thompson and Stuedemann, 1993).

Three classes of neurotropic alkaloids are known from *L. arundinaceum–N. coenophialum* symbiota. The ergot alkaloids, particularly ergopeptines such as ergovaline, are believed to be principally responsible for symptoms of tall fescue toxicosis (Panaccione and Schardl, 2003). Peramine, probably derived from prolyl-arginine, is an insect feeding deterrent (Rowan, 1993). Lolines are the most abundant of the alkaloids and have potent, broad-spectrum insecticidal activity (Riedell et al., 1991).

Sequence analysis of *tub2* (Figure 24.3) and *tef1* (Moon et al., 2004) indicates that *N. coenophialum* is a complex hybrid (Tsai et al., 1994). Three *tub2* alleles and two *tef1* alleles were identified. Also, *N. coenophialum* tends to have multiple allozyme alleles (Leuchtmann and Clay, 1990) and multiple microsatellite alleles (Moon et al., 1999), whereas only single alleles typify the sexual (therefore haploid) *Epichloë* spp. The three *tub2* alleles indicated different *Epichloë* spp. as ancestors. However, one of the ancestors has no close relationship to any known *Epichloë* sp., but is related to the ancestor of three

other hybrid species, *N. occultans* and the two undescribed taxa FaTG-2 and FaTG-3 (*F. arundinacea* endophyte taxonomic groups 2 and 3, where FaTG-1 = *N. coenophialum*) (Christensen et al., 1993; Moon et al., 2000). The clade with these sequences is designated the LAE (*Lolium*-associated endophytes) clade (Moon et al., 2004). The other two ancestors of *N. coenophialum* appear to be *E. festucae* and *E. typhina*, the latter being similar to the genotype associated with *P. nemoralis* (Moon et al., 2004; Tsai et al., 1994).

The hybrid origin of *N. coenophialum* accounts for its heteroploid status, reflected also in large genome size, compared with those of the haploid species *E. festucae* and *E. typhina* (Kuldau et al., 1999). The genome sizes of the two sexual species were estimated at 29 ± 4 Mb, based on quantitative Southern blot analysis of the *tub2* gene. The *N. coenophialum* genome size was estimated at 57 ± 7 Mb. Based on electrophoretic karyotype analysis, *N. coenophialum* was estimated to have 13 chromosomes, including three very large chromosomes (ca. 9.6 Mb) and one very small chromosome (ca. 0.4 Mb), with a total estimated genome size of 61.2 Mb. Thus, quantitative Southern and electrophoretic karyotype analyses gave similar genome size estimates for *N. coenophialum*.

24.3.3 *Neotyphodium lolii* (Latch et al.) A.E. Glenn et al.

This species is a common endophyte of perennial ryegrass (*Lolium perenne* subsp. *perenne*) and was described as an *Acremonium* species (Latch et al., 1984) shortly after the formal description of *A. coenophialum*. The presence of the endophyte was associated both with the livestock toxicosis known as ryegrass staggers (Fletcher and Harvey, 1981; Gallagher et al., 1981) and with protection from insects such as the otherwise devastating Argentine stem weevil (*Listronotus bonariensis* Kuschel). The *L. perenne–N. lolii* symbiosis is a prevalent feature of livestock agriculture in New Zealand, but indolediterpene alkaloids (lolitrems) inhibit high-conductance potassium channels in smooth muscle (Knaus et al., 1994). This, and perhaps effects on the central nervous system, causes ryegrass staggers. Tremorgens structurally related to lolitrems are produced by *Claviceps paspali* F. Stevens & J.G. Hall (ergot of *Paspalum* species) and cause paspalum staggers in livestock (Cole et al., 1977). Related compounds are also produced by other fungi such as *Penicillium paxilli*, from which a cluster of lolitrem biosynthesis genes have been identified (McMillan et al., 2003). In addition to lolitrems, *L. perenne–N. lolii* symbiota may possess peramine and ergot alkaloids. Peramine is considered crucial to combating the Argentine stem weevil (Prestidge et al., 1985). Strains of *N. lolii* have been identified that produce little or no lolitrems or ergot alkaloids, and they are currently being deployed in *L. perenne* cultivars, particularly in New Zealand.

Genetic analysis indicates that *N. lolii* is a haploid, and its phylogenetic relationships indicate derivation from *E. festucae* (Schardl et al., 1994). Despite the close genetic relationship, attempts to mate *N. lolii* as a male to stromata of mat-1 and mat-2 *E. festucae* have consistently failed (Moon et al., 2004). This and the absence of stromata on *L. perenne–N. lolii* symbiota indicate that *N. lolii* is completely asexual.

The *N. lolii* genome was estimated at 30.6 Mb, based on summed sizes of its eight chromosomes observed in electrophoretic karyotyping (Kuldau et al., 1999).

24.3.4 *Neotyphodium lolii* × *Epichloë typhina*

Two endophyte isolates (designated Lp1 and Lp2) from a *L. perenne* population in southern France had similar genotypes indicative of hybrid origin (Christensen et al., 1993; Schardl et al., 1994; Collett et al., 1995). This species has not been formally described, but has been tentatively designated LpTG-2 (*L. perenne* endophyte taxonomic grouping 2, where LpTG-1 = *N. lolii*) (Christensen et al., 1993). Results of analysis of three sequenced loci, eight isozyme loci, and eight microsatellite loci are consistent with its inferred *N. lolii* ×

E. typhina hybrid origin (Schardl et al., 1994; Collett et al., 1995; Moon et al., 2004). The mitochondrial genome appears to be derived from *N. lolii*, and only an *E. typhina* rDNA sequence has been identified in sequence analysis of the internal transcribed spacers (Schardl et al., 1994). The rDNA is unique in that it comprises scores of tandem repeats of an operon encoding three of the four ribosomal RNAs and is subject to rapid concerted evolution by gene conversion. Thus, although two rDNA loci were apparently retained in the hybrid, interlocus gene conversion appears to account for the predominance of the *E. typhina*-derived sequence (Ganley and Scott, 1998, 2002). This phenomenon can account for the common observation that rDNA sequences seldom indicate the hybrid origins indicated by sequences of single-copy genes in many *Neotyphodium* spp. (Schardl et al., 1994; Tsai et al., 1994). Both genome size estimations and electrophoretic karyotyping support the inference that isolate Lp1 is a diploid or near-diploid hybrid, with 13 chromosomes totaling 55 ± 7 Mb (Murray et al., 1992; Kuldau et al., 1999).

The *N. lolii* × *E. typhina* isolate Lp1 has proven to be of great importance in endophyte molecular biological studies. This was the first *Neotyphodium* sp. isolate to be transformed with exogenous DNA (Murray et al., 1992). Subsequently, the transformation system was applied to analysis of ergot alkaloid biosynthesis genes. First, *lpsA*, a homolog of the suspected lysergyl peptide synthetase gene of *Cl. purpurea*, was identified in isolate Lp1 (Panaccione et al., 2001). The *lpsA* gene was mutated by introduction of a selectable marker (*hph*, providing hygromycin B resistance) within its coding sequence. The resulting mutant was still capable of producing lysergic acid and clavine alkaloids, but was unable to produce ergovaline, the principal cyclic peptine derivative of lysergic acid in *Neotyphodium* spp. (Panaccione et al., 2001, 2003). The *dmaW* gene for dimethylallyltryptophan synthase was also identified and similarly disrupted in isolate Lp1 (Wang et al., 2004). This mutant was incapable of producing ergovaline, lysergic acid, and the precursor chanoclavine I. Introduction of the *dmaW* homolog from *Claviceps fusiformis* Loveless restored ergovaline production. These results confirmed that dimethylallyltryptophan synthase is the determinant step in the ergot alkaloid biosynthetic pathway.

24.3.5 *Neotyphodium occultans* Moon et al.

In the late 19th and early 20th centuries a number of studies focused on the seed fungus of *Lolium temulentum* L., one of the annual ryegrasses and long regarded as a toxic. Early studies by Guérin (1898), Vogel (1898), and Freeman (1904) established the systemic nature of the fungus and the possible mutualistic nature of the symbiosis. Freeman (1904) carefully traced the entire life cycle from growth in leaf, stem, and floral and seed tissues through invasion of the ovary, ovule, and embryo, and subsequent growth concomitant with germination and seedling growth. With slight variation, this process of coordinated plant and fungus growth holds for all poöid–epichloë symbiota except for the additional development of the fruiting bodies (stromata) by *Epichloë* species. Interestingly, despite the later associations of *N. coenophialum* and *N. lolii* with toxicoses to livestock, no such role has been substantiated for *N. occultans*. (Instead, annual ryegrass toxicoses have been associated with bacterial infections [McKay and Ophel, 1993; Riley et al., 2003].) An unusual aspect of *N. occultans* is that it is unculturable separate from host tissues. Thus, *N. occultans* is known only from the systemic hyphae in plants, the mycelial mat beneath the seed coat, and hyphae penetrating the embryonic axis, permitting vertical transmission.

Several annual *Lolium* spp. and subspecies of *L. perenne* are frequently symbiotic with *N. occultans*, which may provide some biological protection to these hosts. Alkaloid profiles of endophyte-symbiotic annual ryegrasses indicated insecticidal loline alkaloids,

similar to those in *L. arundinaceum–N. coenophialum* symbiota (TePaske et al., 1993). Later, *N. occultans* was characterized as an interspecific hybrid, with an *E. bromicola* and an LAE clade ancestor (Moon et al., 2002).

24.3.6 *Neotyphodium uncinatum* (W. Gams et al.) A.E. Glenn et al.

This is the most common endophyte in European meadow fescue (*Lolium pratense* [Huds.] Darbysh. = *Festuca pratensis* Huds.), and a majority of the grass throughout Europe is symbiotic with *N. uncinatum* (Craven et al., 2001a). The endophyte produces very high levels of loline alkaloids (Bush et al., 1997; Craven et al., 2001a), which serve as a broadly active insecticide (Riedell et al., 1991). In addition, the endophyte is associated with improved resistance to water stress (Malinowski et al., 1997).

Among the four classes of alkaloids specifically associated with grass–epichloë symbiota, *L. pratense–N. uncinatum* symbiota are only known to produce lolines (Bush et al., 1997), and at up to 2% dry mass of leaf tissues, the lolines are especially abundant in these symbiota (Bush et al., 1997; Craven et al., 2001a). Up through the 1990s the lolines remained the only class never reported from fungal cultures. Then, a minimal nutrients medium was devised for fermentation cultures of *N. uncinatum*, in which the fungus produced lolines at levels comparable with those produced *in symbio* (Blankenship et al., 2001). Although complex media such as potato dextrose broth would support greater biomass production, the fungus produced no detectable lolines in complex medium. Furthermore, lolines most rapidly accumulated as the fungus entered stationary phase. Fermentation cultures of *N. uncinatum* have proven especially useful for the investigation of loline alkaloid biosynthesis and the associated genes involved (Spiering et al., 2002).

Molecular phylogenetic analysis indicates a hybrid origin of *N. uncinatum* (Craven et al., 2001a). The *tub2* gene indicates a relationship to *E. typhina* and is very similar to the sequence of the *E. typhina* ancestor of *N. coenophialum*. The *tef1* gene indicates an *E. bromicola* ancestor. Analysis of *act1* (the gamma-actin gene) indicates two alleles, one with the *E. typhina* relationship and the other with the *E. bromicola* relationship. A characteristic of hybrid *Neotyphodium* species is the presence of multiple alleles for some loci, but apparently the evolution of *N. uncinatum* from its hybrid ancestor has involved loss of many redundant alleles. This is evident for *tub2* and *tef1*. Likewise, analyses of allozymes (Christensen et al., 1993) and microsatellites (Moon et al., 2004) indicate that this species is nearly haploid and has very few redundant alleles compared with other hybrids.

The conidial morphology typifying *N. uncinatum* is unique among *Neotyphodium* spp. Rather than the navicular or near-elliptical shape of typical *Neotyphodium* conidia, *N. uncinatum* spores are longer and often hook shaped, hence the specific epithet (Gams et al., 1990). A morphological variant having equally long but straighter spores correlates with a slight difference in allozyme profile (Christensen et al., 1993). The *N. uncinatum* conidium is formed singularly from a conidiogenous cell to which it tends to adhere. The spore is therefore holoblastic, whereas other *Neotyphodium* spp. form enteroblastic spores. Nevertheless, given its relationship with *Neotyphodium* and *Epichloë* species, it appears most prudent to retain classification of *N. uncinatum* in the genus despite the atypical spore morphogenesis.

24.3.7 *Neotyphodium siegelii* Craven et al.

A screen by K. Hignight (Advanta Seeds Pacific) of a plant introduction (P.I.) of meadow fescue (*L. pratense*) for endophytes compatible with *L. perenne* led to the identification of this new species (Craven et al., 2001a). A follow-up survey of that P.I. and other

accessions from throughout Europe failed to uncover another *N. siegelii* isolate. Because the original seed that harbored *N. siegelii* was destroyed in the isolation process, it is not known for certain that the endophyte was truly a natural symbiont of meadow fescue; conceivably, it could have been from a rare contaminant of the P.I. seed lot. To help assess the plausibility of the hypothesis that *N. siegelii* is a *L. pratense* endophyte, it was (re)introduced into *L. pratense* plants. Its stability and seed transmissibility in *L. pratense* (Craven et al., 2001a) support the hypothesis, given the host specificity that typifies epichloë endophytes. However, compatibility with *L. perenne* (Braman et al., 2002) and *L. arundinaceum* (C.L. Schardl, unpublished data) indicates that *N. siegelii* has a broader potential host range than *N. uncinatum* (Christensen et al., 2000).

Like *N. uncinatum*, *N. siegelii* has the potential to produce high levels of lolines (Craven et al., 2001a). These alkaloids have been identified in both its *L. perenne* and *L. pratense* symbiota. Although constitutive levels of lolines in *L. pratense–N. siegelii* symbiota were much lower than in *L. pratense–N. uncinatum* symbiota, both showed dramatically elevated levels 11 days after the plants were clipped. Final total loline concentrations for both were 1.9 to 2.0% lolines based on leaf and pseudostem dry mass, respectively. The induction of loline alkaloid synthesis upon clipping (mock herbivory) suggests an adaptation to combat herbivorous insects. Yet in contrast to induced plant defenses against herbivores, this is a response of a mutualistic fungus to damage of the symbiotum.

N. siegelii shares more than a host and a biochemical similarity with *N. uncinatum*, being also a hybrid with an *E. bromicola* ancestor (Craven et al., 2001a). However, the other ancestor of *N. siegelii* is *E. festucae*. It is tempting to postulate that the shared ancestor may have contributed the loline alkaloid production genes. However, no loline-producing *E. bromicola* isolate has yet been identified.

Conidia of *N. siegelii* are morphologically and developmentally typical of those of most epichloë endophytes and, therefore, differ from those of the atypical species *N. uncinatum* (Craven et al., 2001a). The *N. siegelii* conidia are larger than those of haploid *Neotyphodium* and *Epichloë* spp. (Table 24.1), probably reflecting the diploid or near-diploid nature of the *N. siegelii* genome.

24.3.8 *Neotyphodium huerfanum* (J.F. White et al.) A.E. Glenn et al.

The first *Neotyphodium* species from *Festuca arizonica* Vasey was described as *N. huerfanum*. Aside from a reddish brown color on the reverse of Potato Dextrose Agar (PDA) cultures, this species was recognized as being highly similar to *N. typhinum* (White et al., 1987). Indeed, sequence data from the *ex type* isolate indicate a very close relationship to the *E. typhina* genotypes associated with *P. nemoralis* (Moon et al., 2004). Consideration may be given to relegating *N. huerfanum* to a variety status within *N. typhinum*.

Other isolates from *F. arizonica* have been classified as *N. starrii* or *N. tembladerae*, as discussed below.

24.3.9 *Neotyphodium starrii* (J.F. White et G. Morgan-Jones) A.E. Glenn et al.

The type of *N. starrii* was on *Festuca subulata* Trin., but *ex type* material has not been deposited in culture collections for evaluation. The species description encompasses some endophytes of *F. arizonica* and *Bromus anomalus* Rupr. ex E. Fourn. (White and Morgan-Jones, 1987b). Only the ex *F. arizonica* isolate has been investigated by sequence analysis, whereby the rDNA ITS1-2 region was identical to that of *N. huerfanum* (An et al., 1992). The close resemblance of *N. starrii* to *N. huerfanum* (White et al., 1993) and the absence of live *ex type* material for further analysis make use of this binomial problematic.

24.3.10 *Neotyphodium tembladerae* Cabral et al.

This is the first *Neotyphodium* sp. to be described from the southern hemisphere (specifically Argentina) (Cabral et al., 1999). Of interest was its identification on multiple, distantly related hosts, including *Poa huecu* Parodi (on which was the type), *Festuca argentina* (Speg.) Parodi, and *F. hieronymi* Hack. An isolate considered very similar to *N. tembladerae* was also obtained from *Bromus setifolius* J. Presl. from Argentina (White et al., 2001). The symbiosis with *P. huecu* is associated with the sometimes lethal huecú toxicosis suffered by livestock that graze this endophyte-infected grass. Associated stagger symptoms suggest the involvement of indolediterpene alkaloids such as the lolitrems (Miles et al., 1998).

Analysis of *tub2* and *tef1* sequences indicate that the *N. tembladerae ex type* isolate is a hybrid of *E. typhina* and *E. festucae* (Moon et al., 2002). The *E. typhina* genotype is similar to those on *P. nemoralis*. Identical sequences have been obtained in an analysis of an isolate from *F. arizonica*, the morphology of which is also consistent with classification as *N. tembladerae* (Moon et al., 2004). The broad host and geographic range of *N. tembladerae* is intriguing, and there are several possible explanations. Contagious spread of the species is conceivable, although *Neotyphodium* species are notoriously difficult to introduce into hosts except by vertical transmission or by highly invasive laboratory protocols (Latch and Christensen, 1985; Johnson-Cicalese et al., 2000). Another possibility is that the hybrid was in a common ancestor of its hosts. But this seems highly unlikely given that the hosts span at least two and possibly more genera. A third possibility is that multiple hybrids of *E. festucae* and *E. typhina* have given genotypically and phenotypically similar endophytes in distinct hosts. This last possibility is highly plausible. Both *E. festucae* and *E. typhina* genomes are frequent components of hybrid endophytes. The *E. festucae* components are likely to appear very similar because *E. festucae* shows little variation in *tub2* and *tef1* sequences. In contrast, *E. typhina* is highly diverse; however, genotypes related to *E. typhina* in *P. nemoralis* are most commonly involved in evolution of both nonhybrid and hybrid *Neotyphodium* spp.

24.3.11 *Neotyphodium chisosum* (J.F. White et G. Morgan-Jones) A.E. Glenn et al.

This is an endophyte isolate from a *Stipa eminens* Cav. plant obtained in Cloudcroft, NM (White and Morgan-Jones, 1987a). There is some confusion in the literature because of chemical profiling of the related grass *Achnatherum robustum* (Vasey) Barkworth (= *Stipa robusta* (Vasey) Scribn.) from the same geographical area (TePaske et al., 1993). The latter has not been characterized by molecular phylogenetics, but the former recently was subjected to *tub1* and *tef1* sequencing, as well as microsatellite analysis (Moon et al., 2004). Interestingly, *N. chisosum* is the only one of two interspecific hybrids so far identified with three ancestors (the other being *N. coenophialum*, discussed above). In the case of *N. chisosum*, the ancestors are related to *E. bromicola*, *E. typhina*, and *E. amarillans*. The *E. bromicola* relationship is distant to that of extant isolates of that species, suggesting either that this genome represents an early introduction into the *S. eminens* lineage or that it is from an *Epichloë* species as yet unidentified. The *E. typhina* relationship is close to that of *E. typhina* associated with *Poa pratensis* L. This is an *E. typhina* clade that is sister to the *P. nemoralis*-associated genotypes and appears to have been involved in at least one other hybridization (with *E. festucae* to give *N. australiense* Moon et al.) and in the origin of an endophyte of *Poa sylvestris* A. Gray. The *E. amarillans* genotype is related but not identical to those sampled to date.

24.3.12 *Neotyphodium aotearoae* Moon et al.

This represents the first *Neotyphodium* species so far identified from a grass indigenous to New Zealand, and the species also occurs in Australia in association with the same host, *Echinopogon ovatus* (G. Forst) P. Beauv. (Moon et al., 2002). This endophyte grows very slowly in culture and does not produce aerial hyphae or sporulate. It is vertically seed transmitted in *Ech. ovatus* and may occur in other *Echinopogon* species from Australia. Loline alkaloids are produced in plants symbiotic with *N. aotearoae* (Miles et al., 1998).

Sequence analysis indicates that *N. aotearoae* is probably nonhybrid and is not closely related to any extant *Epichloë* species so far identified. However, it groups within the *Epichloë* clade (Figure 24.3). Furthermore, a genotype very similar to that of *N. aotearoae* appears to be one ancestor of the hybrid endophyte *Neotyphodium melicicola* Moon et al. This relationship strongly suggests that in the southern hemisphere there is or recently has been an *Epichloë* species from which *N. aotearoae* and the related genome in *N. melicicola* were derived.

24.3.13 *Neotyphodium australiense* Moon et al.

Australian *Ech. ovatus* plants may harbor either *N. aotearoae* or the hybrid endophyte *N. australiense* (Moon et al., 2002). Compared with *N. aotearoae*, *N. australiense* grows quickly and sporulates well. It is possible that this endophyte also produces indolediterpenes associated with staggers (Miles et al., 1998), a toxic symptom associated with *Ech. ovatus* in Australia and similar to ryegrass staggers. The ancestry of *N. australiense*, based on *tef1* and *tub2* phylogenies, is *E. festucae* × *E. typhina*, where the *E. typhina* component is related to that in *P. pratensis* (Moon et al., 2002).

24.3.14 *Neotyphodium melicicola* Moon et al.

Another southern hemisphere endophyte, this species is found in *Melica decumbens* Thunb. from South Africa. The grass is called *dronkgras* in Afrikaans, in reference to symptoms of livestock that graze it (Meredith, 1955; Botha and Naude, 2002). The malady is likely due to endophyte alkaloids, perhaps indolediterpenes. The two *tub2* alleles, and some multiple allele microsatellite loci, suggest a hybrid origin for *N. melicicola* (Moon et al., 2002). Based on *tub2* phylogeny, the ancestors appear to be *E. festucae* and a close relative of *N. aotearoae*. Only a single *tef1* allele has been detected in *N. melicicola*, and its sequence confirms a relationship with *N. aotearoae*. Presumably, the *E. festucae*-derived allele from the ancestral hybrid has been lost, or mutations have prevented its amplification by the PCR approach used to identify *tub2* alleles.

The relationship of an *N. melicicola* genome component to *E. festucae* is intriguing in that it rounds out the geographical distribution of *E. festucae* and its hybrid derivatives to five continents: North and South America, Australia, Africa (both northern and southern), and Europe.

24.3.15 *Neotyphodium inebrians nom. provis.* and *N. gansuense* C.J. Li

A common endophyte of *Achnatherum inebrians* (Hance) Keng has been included in molecular phylogenetic studies, where it is provisionally called *N. inebrians* (Moon et al., 2004). The host is native to northwestern China, where it is called "drunken horse grass." Animals grazing this grass suffer stupor much like that of animals in the southwestern U.S. that graze "sleepy grass," *Ach. robustum* with an apparently toxic endophyte (Petroski et al., 1992). In both cases, the endophyte-infected grass accumulates high levels of lysergic acid amide and ergonovine, two simple ergot alkaloids. Up to 2900 µg/kg dry mass of these ergot alkaloids was measured in *Ach. inebrians-N. gansuense* symbiota (Miles et

al., 1996). Another reason for special interest in *'N. inebrians'* is its phylogenetic position, representing a clade distinct from that of any *Epichloë* sp. or any other nonhybrid endophyte so far identified (Figure 24.3).

Recently a new endophyte species was identified in *Ach. inebrians*, and described as *Neotyphodium gansuense* (Li et al., 2004). As a moderately fast-growing *Neotyphodium* species, which sporulates well under certain conditions, *N. gansuense* is distinct from the slow-growing, nonsporulating *'N. inebrians'*.

24.3.16 Undescribed *Neotyphodium* Species

Numerous additional *Neotyphodium* isolates with unique evolutionary origins have been identified and are listed in Table 24.1 by taxonomic grouping (TG) designations. Among the other undescribed *Neotyphodium* species are a large number of interspecific hybrids (Moon et al., 2004). Table 24.1 lists the nonhybrid species most closely related to contributors to the genomes of each of these hybrids. Based on phylogenetic analysis (Figure 24.3), the origins of several of these seem apparent. As previously mentioned, LpTG-2 is clearly related to *N. lolii* and *E. typhina*, although it is possible that this is actually an *E. festucae* × *E. typhina* hybrid since *N. lolii* is genetically similar to *E. festucae*. This endophyte is unusual in one sense, however, in that both inferred ancestors are associated with the same host, *L. perenne*: *N. lolii* is a common endophyte of this grass, and the closest *E. typhina* relative is the isolate from *L. perenne*.

Hybrid endophytes were identified in *Ach. robustum*, *Festuca altissima* All., *F. paradoxa* Desv., *Hordelymus europaeus* (L.) Harz, *Hordeum bogdanii* Wilensky, *H. brevisubulatum* (Trin.) Link, *Melica ciliata* L., and *Poa autumnalis* Muhl. ex Ell. (Table 24.1, Figure 24.3) (Moon et al., 2004). Also, two distinct hybrid taxa, FaTG-2 and FaTG-3, were isolated from plants identified as *F. arundinacea* (*L. arundinaceum*) (Christensen et al., 1993; Tsai et al., 1994), although analysis of chloroplast DNA sequences indicated that their host is probably a species distinct from *L. arundinaceum* (K.D. Craven and C.L. Schardl, unpublished data). In several hosts with hybrid endophytes, nonhybrids were also found. Two of these were mentioned earlier: an *E. bromicola* isolate from *He. europaeus* and *N. lolii* from *L. perenne*. Also, an isolate classified as HbrTG-1 from *H. brevisubulatum* was related to *E. bromicola*, but its mating compatibility has not yet been tested. An endophyte related to, but not interfertile with, *E. festucae* was found in asymptomatic plants of *Festuca obtusa* Bieler. Similarly, an endophyte from *Poa sylvestris* A. Gray was related to *E. typhina*, but attempted matings with *E. typhina* yielded no ascospores. Also related to *E. typhina* were a nonculturable endophyte designated *N. typhinum* var. *canariense* from (Moon et al., 2000) *Lolium edwardii* H. Scholz et al. (A. Stewart, personal communication), and the aforementioned *N. huerfanum* from *F. arizonica*. Mating tests with the former would be unfeasible, and the latter has not yet been tested for interfertility with *E. typhina*.

An informative morphological feature of *Neotyphodium* and *Epichloë* spp. is the size and dimensions of conidia. The sexual species tend to have conidia of very similar size and shape, whereas much more variation is seen among asexual *Neotyphodium* spp. The hybrids consistently have larger spores than sexual and asexual nonhybrids (Table 24.1), supporting the hypothesis that spore size may reflect genome size in the epichloë endophytes (Kuldau et al., 1999).

24.3.17 *Acremonium chilense* G. Morgan-Jones et J.F. White

This species (Morgan-Jones et al., 1990) is not closely related to *Neotyphodium*, but a typical *Acremonium* species of section *Simplex.* Characteristic are the rod-shaped conidia, the fast growth in culture, and the increased proliferation, particularly in more senescent tissues of

Table 24.1 Species of *Epichloë* Endophytes and Their Characteristics

Species	Spore Size[a] (μm)	Pedigree[b]	Hosts	Geographic Origin[c]
Epichloë amarillans	4.5 ± 0.7 × 1.9 ± 0.2	Eam	*Agrostis* spp., *Sphenopholis* spp.	N. America
E. baconii	4.4 ± 0.6 × 1.8 ± 0.3	Eba	*Agrostis* spp., *Calamagrostis villosa*	Europe
E. brachyelytri	4.1 ± 0.6 × 2.8 ± 0.3	Ebe	*Brachyelytrum erectum*	N. America
E. bromicola	3.8 ± 0.4 × 2.0 ± 0.3	Ebr	*Bromus* spp.	Europe
E. bromicola	5.2 ± 0.3 × 3.5 ± 0.2	Ebr	*Hordelymus europaeus*	Europe
E. clarkii	4.4 ± 0.4 × 1.9 ± 0.1	ETC	*Holcus lanatus*	Europe
E. elymi	4.0 ± 0.4 × 2.2 ± 0.2	Eel	*Elymus* spp.	N. America
E. elymi	nt	Eel	*Bromus kalmii*	N. America
E. festucae	4.7 ± 0.6 × 2.2 ± 0.3	Efe	*Festuca* spp., *Lolium* spp.	Europe
E. glyceriae	5.0 ± 0.2 × 2.6 ± 0.2	Egl	*Glyceria striata*	N. America
E. sylvatica	5.0 ± 1.1 × 2.0 ± 0.5	ETC	*Brachypodium sylvaticum*	Europe
E. typhina	4.9 ± 0.4 × 2.5 ± 0.4	ETC	*Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Brachypodium pinnatum*, *Bp. phoenicoides*, *Lolium perenne*, *Phleum pratense*, *Poa* spp., *Puccinellia distans*	Europe
Neotyphodium aotearoae	no	Nao	*Echinopogon ovatus*	New Zealand, Australia
N. australiense	6.4 ± 0.5 × 3.7 ± 0.4	Efe, Ety	*Ech. ovatus*	Australia
N chisosum	7.0 ± 2.0 × 3.3 ± 0.8	Eam, Ebr, ETC	*Achnatherum eminens*	N. America
N. coenophialum	8.2 ± 1.4 × 2.2 ± 0.5	Efe, ETC, LAE	*Lolium arundinaceum*	Europe
N. huerfanum	3.0 ± 1.0 × 2.5 ± 0.5	ETC	*Festuca arizonica*	N. America
'N. inebrians'	no	Nin	*Achnatherum inebrians*	Asia
N. lolii	no	Efe	*L. perenne* subsp. *perenne*	Europe
N. melicicola	7.1 ± 0.9 × 4.4 ± 0.4	Efe, Nao	*Melica decumbens*	S. Africa
N. occultans	no	Ebr, LAE	*L. perenne* subspp. (annual)	Europe
N. siegelii	6.8 ± 0.5 × 3.0 ± 0.3	Ebr, Efe	*Lolium pratense*	Europe
N. tembladerae	6.7 ± 0.7 × 3.7 ± 0.4	Efe, ETC	*Poa huecu*	S. America

Table 24.1 Species of *Epichloë* Endophytes and Their Characteristics (Continued)

Species	Spore Size[a] (μm)	Pedigree[b]	Hosts[c]	Geographic Origin
N. tembladerae	7.1 ± 0.4 × 4.0 ± 0.4	Efe, ETC	*Festuca arizonica*	N. America
N. typhinum var. *canariense*	no	ETC	*Lolium edwardii*	Canary Islands
N. uncinatum	Variable	Ebr, ETC	*L. pratense*	Europe
N. sp. AroTG-1	7.7 ± 0.8 × 3.9 ± 0.4	Eel, Efe	*Achnatherum robustum*	N. America
N. sp. FaTG-2	6.9 ± 0.7 × 2.7 ± 0.4	Efe, LAE	*Lolium* sp.	S. Europe, N. Africa
N. sp. FaTG-3	7.9 ± 0.9 × 2.4 ± 0.5	ETC, LAE	*Lolium* sp.	S. Europe, N. Africa
N. sp. FalTG-1	7.1 ± 0.5 × 3.0 ± 0.3	Ebr, ETC	*Festuca altissima*	Europe
N. sp. FobTG-1	5.4 ± 0.4 × 3.4 ± 0.4	Efe	*Festuca obtusa*	N. America
N. sp. FpaTG-1	6.2 ± 0.6 × 3.6 ± 0.4	Eam, ETC	*Festuca paradoxa*	N. America
N. sp. HeuTG-2	8.3 ± 0.6 × 3.5 ± 0.2	Ebr, ETC	*He. europaeus*	Europe
N. sp. HboTG-1	8.3 ± 1.1 × 3.6 ± 0.3	Eam, Eel	*Hordeum bogdanii*	Asia
N. sp. HboTG-2	6.5 ± 0.8 × 4.0 ± 0.4	Ebr, ETC	*H. bogdanii*	Asia
N. sp. HbrTG-1	5.4 ± 0.4 × 3.3 ± 0.3	Ebr	*Hordeum brevisubulatum*	Asia
N. sp. HbrTG-2	7.0 ± 0.7 × 3.4 ± 0.3	Ebr, ETC	*H. brevisubulatum*	Asia
N. sp. LpTG-2	7.1 ± 0.4 × 3.1 ± 0.2	Efe, ETC	*L. perenne* subsp. *perenne*	Europe
N. sp. MciTG-1	6.2 ± 0.5 × 3.5 ± 0.3	ETC, Nin	*Melica ciliata*	Europe
N. sp. PauTG-1	7.9 ± 0.6 × 3.1 ± 0.4	Eel, ETC	*Poa autumnalis*	N. America
N. sp. PsyTG-1	5.0 ± 0.5 × 2.9 ± 0.3	ETC	*Poa sylvestris*	N. America

[a] Spore sizes for *Epichloë* spp., *N. chisosum*, and *N. huerfanum* were taken from original taxonomic descriptions. Measurements from *E. glyceriae*, *N. huerfanum*, and *N. chisosum* were originally reported as a size range, but the median and range have been shown above for consistency. no = data not available when spores were not observed; nt = data not available when isolate was not tested.

[b] The most closely related *Epichloë* species are indicated, except where an ancestor is more closely related to *N. aotearoae* or '*N. inebrians*.' Abbreviations: Eam = *Epichloë amarillans*; Ebr = *E. bromicola*; Eel = *E. elymi*; Efe = *E. festucae*; ETC = *E. typhina* complex (including *E. typhina*, *E. clarkii*, and *E. sylvatica*); LAE = *Lolium*-associated clade (see Figure 24.3); Nao = *Neotyphodium aotearoae*; Nin = *N. inebrians*.

[c] The original geographical origin of the host species is indicated.

the host, which are typical of saprotrophic fungi. Similar *Acremonium* species have also been regularly isolated from *Festuca paniculata* (L.) Schinz & Thell. and annual *Lolium* species (Naffaa et al., 1998). Glenn et al. (1996) placed this species into the new genus *Neotyphodium*, together with the others mentioned above. However, the treatment that would best reflect the characteristics of this species is to retain the species in *Acremonium*.

24.4 CONCLUSIONS

There are several compelling reasons for interest in the epichloë–grass symbioses. These represent the only example in which a single genus of symbiont spans the continuum from pathogenic to mutualistic associations (considering *Epichloë* and the anamorph genus *Neotyphodium* to be essentially a single genus). The pleiotropic symbioses can in some sense be regarded as intermediate between the two, even though many of these also appear to be mutualisms (Schardl, 2001). Also of great interest is the range of benefits provided by the endophytes (reviewed in Malinowski and Belesky, 2000; Clay and Schardl, 2002; Schardl et al., 2004b). The antiherbivore activities of the alkaloids provide important benefits, but additional benefits in growth, nutrient acquisition and utilization, and tolerance to heat and drought stress have been documented for several epichloë–grass symbiota.

The evolutionary relationships of epichloë endophytes are particularly intriguing. Most of the *Epichloë* species are specific for individual host genera or related genera. The exception is *E. typhina*, which, however, may comprise several host-adapted populations. Similarly, most *Neotyphodium* spp. also are confined to individual host species or genera. But interestingly, there is no consistent relationship between the hosts of *Neotyphodium* spp. and their closest *Epichloë* spp. relatives. In fact, some endophytes seem to have lost the ability to fruit concomitant with or following colonization of new hosts (host jumps), perhaps indicating that some hosts are nonpermissive for stroma development. Furthermore, the majority of *Neotyphodium* spp. surveyed so far are interspecific hybrids.

There is some suggestion that certain hybrids arose following host jumps in which the endophyte was rendered asexual. Possible examples are indicated in the relationship of *N. lolii* and the hybrid LpTG-2, and in the relationship of the nonhybrid HbrTG-1 and the hybrid HbrTG-2. However, such examples are rare, possibly because hybrids tend to dominate host populations (as in *He. europaeus*, *L. arundinacea*, and *L. pratense*) (Moon et al., 2004) and may, therefore, replace their nonhybrid asexual ancestors over evolutionary time. Also, this scenario supposes that hybridization was somatic rather than by interspecific mating. Indeed, somatic hybridization seems both feasible and likely for these endophytes, since interspecific heterokaryons can be produced experimentally (Chung and Schardl, 1997b). Additionally, the observation that some hybrids — *N. coenophialum* and *N. chisosum* — have contributions from three different *Epichloë* species supports a somatic hybridization scenario. In the evolution of each of these endophytes, one hybridization would have brought together two ancestral genomes, and the resulting two species, diploid hybrid would almost certainly have been asexual (because no heteroploid sexual isolates have been observed among the *Epichloë* species). Vertical transmission would have maintained the hybrid until a host plant or seed was also infected by another *Epichloë* species. Then the two-part hybrid would hybridize further with the newcomer to develop the three-part hybrid. In the case of *N. coenophialum* at least, the result has not been a triploid, presumably because chromosomes and chromosome segments have been lost. Rather, this endophyte has approximately twice the genome size of the *Epichloë* species, but with some gene copies from each of its three ancestors.

The prevalence of hybrid endophytes within many host populations and among host species suggests that there may be a strong selective advantage conferred by interspecific hybridization. Among the advantages may be (1) ameliorating inherent fitness costs of clonality (Rice, 2002); (2) acquisition of beneficial genes, such as alkaloid biosynthesis genes, which may have evolved or may have been acquired in other systems; and (3) enhanced evolutionary potential, perhaps better enabling the endophyte to keep pace as the host species evolve, especially considering that many of the host grasses are also interspecific hybrids.

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25

Evolutionary Development of the Clavicipitaceae

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25.1 OVERVIEW OF CLAVICIPITACEAE

Clavicipitaceae is a fungal family in the order Hypocreales (Ascomycota). It is largely composed of plant biotrophs and pathogens of arthropods and *Elaphomyces* (truffles). Clavicipitalean fungi have many unseen and often unstudied impacts in plant and herbivore communities. They are important in natural biological control of herbivores of plants by deterring herbivore feeding on plants (Clay, 1988) and have been shown to defend plants from fungal diseases and abiotic stresses (White et al., 2002). The entomopathogenic clavicipitaleans play important functions in regulating arthropod populations in their natural environment. The diseases caused by these arthropod pathogens can be of epidemic proportions (Evans and Samson, 1982; Hywel-Jones, 1997).

Previously, Clavicipitaceae held a higher taxonomic position as a distinct order from the Hypocreales (Nannfeldt, 1932; Rogerson, 1970). However, more recent molecular analyses have shown Clavicipitaceae to be derived from within Hypocreales (Spatafora and Blackwell, 1993; Rehner and Samuels, 1995).

Typical morphological characters associated with clavicipitalean fungi are the development of perithecia usually associated with a fleshy, brightly colored stroma; unitunicate asci with a thickened tip that is perforated by a cylindrical pore; and multiseptate filiform ascospores. Anamorphs of Clavicipitaceae are largely considered to be hyphomycetes

with the exception of *Aschersonia* and *Munkia*, both of which produce pycnidial-like cavities within stromata. Multiple phylogenetic studies have supported the monophyly of the family (Spatafora and Blackwell, 1993; Rehner and Samuels, 1995; Artjariyasripong et al., 2001).

25.2 HISTORICAL AND ECONOMIC IMPACTS OF CLAVICIPITACEAE

The fungal family Clavicipitaceae shares a turbulent history with agricultural humans. The most well known and studied of these interactions, the disease ergotism, is caused by the clavicipitalean fungus *Claviceps purpurea*. This disease has often been referred to as St. Anthony's Fire or Holy Fire (Hudler, 1998). *C. purpurea* has been implicated in playing a major role in some of humanity's darkest periods, including the Black Plague and the witch trials of Salem (Caporael, 1976; Matossian, 1989). *C. purpurea*, along with other species of this genus, produce sclerotia that replace the seeds of numerous cereal crops. These sclerotia were often accidentally or intentionally included to increase bulk along with rye grains, a staple crop, and consumed. People who consumed the contaminated rye were often inflicted with bouts of hysteria, dementia, and hallucinations. In severe cases of ergotism, victims developed gangrene and lost some of their limbs or died due to the vasoconstrictive properties of the ergot alkaloids. Others died from infections that accompanied the chemically induced gangrene.

Some clavicipitalean species that live endophytically within grasses (e.g., *Epichloë*, *Balansia*) also produce alkaloids that cause symptoms in mammalian herbivores similar to those experienced by humans that consume *Claviceps* sclerotia. *Neotyphodium tembladerae* (an anamorph of the genus *Epichloë*) has been shown to produce the alkaloids ergovaline and peramine. These alkaloids can cause a type of drunken staggers, or even death in herbivores that consume infected grasses (White et al., 2001).

Some of the same compounds that make these fungi so dangerous to consume also provide the fungus with properties that have been found to be beneficial to humans. Midwives of Colonial America would often grind up the sclerotia for a liquid concoction to be consumed by pregnant women to induce labor (Barger, 1931). The Jívaro tribe of the Amazon used another clavicipitalean, *Balansia cyperi*, to produce a similar effect (Lewis and Elvin-Lewis, 1990).

Modern medicine has also found the powerful compounds produced by clavicipitalean fungi to be useful in various treatments. Clinical studies have shown the effectiveness of *Cordyceps* species in the treatment of sexual dysfunction (Yang et al., 1985) and breast and lung cancer (Zhou and Lin, 1995). Metabolic products of the clavicipitalean rice pathogen, *Ustilaginoidea virens*, are able to arrest mitotic division of a number of human tumor cell lines (Hutton and White, 1994). Furthermore, modern surgeons are able to perform organ transplants due to the immunosuppresent activity of cyclosporin, which was extracted from *Cordyceps subsessilus* (the teleomorphic state of *Tolypocladium inflatum*; Hodge et al., 1996). Many other taxa within Clavicipitaceae likely contain pharmaceutically useful compounds yet to be discovered.

Clavicipitaleans have also been used by some people for intoxication and to induce spiritual experiences (White et al., 2003). Some historians consider the lysergic acid diethylamide (LSD), contained in ergot sclerotia, to have been used in many ancient sacred rituals such as the Eleusinian mysteries (Wasson et al., 1978), the manna of the Israelites (Merkur, 2000), and the Great Awakening (Matossian, 1989). In the 1960s, Harvard

professors Timothy Leary and Richard Alpert conducted research on the experiences and sensations of drug-induced hallucinations. Among these drugs was LSD. One such experience Leary described as "without question the deepest religious experience of my life" (Forte, 1999, p. 9).

25.3 CLAVICIPITALEAN EVOLUTION

In the following sections we:

1. Develop hypotheses regarding interkingdom host shifts within the family
2. Develop hypotheses regarding the origins of the endophytic lifestyle in Clavicipitaceae
3. Investigate various anamorphic states that exist in the family and their utility in delimiting monophyletic groups
4. Examine clavicipitalean taxa that had not been included in a modern phylogenetic context before

25.3.1 Host Shifting in Clavicipitaceae

The full scope of host substrates occupied by clavicipitaleans is far from realized. However, trends regarding the evolution of interactions between members of Clavicipitaceae and their hosts can certainly be observed. No one hypothesis of host shifting seems to explain host distribution in the entire family, but with the inclusion of more taxa, host substrate patterns will likely become more apparent.

Nikoh and Fukatsu (2000) used the host habitat hypothesis to explain host shifting from *Elaphomyces* (truffles) to Cicadidae (cicadas). According to this hypothesis, host shifts may occur due to overlap in microhabitat or feeding habit (Shaw, 1988). While their conclusions regarding the ancestral host of *Cordyceps* may be overstated due to limitations of sampling, Nikoh and Fukatsu make a strong case for interkingdom host jumps through commingling in soil microhabitats. A strong correlation between fungal relatedness and the microhabitat of members of clade C (Figure 25.1) support the findings of Nikoh and Fukatsu (2000). All taxa of clade C are regularly collected from either the soil (*Tolypocladium cylindrosporus*, *Cordyceps subsessilis* [as *T. inflatum*], and *Chaunopycnis* spp.) or soil-associated organisms (e.g., cicada larvae and truffles).

Scale insects parasitized by entomopathogenic clavicipitaleans (e.g., *Dussiella tuberiformis* and *Torrubiella luteorostrata*) may have provided the avenue for the interkingdom host shift to occur and lead to the eventual evolution of plant biotrophs and symbionts (clade D, Figure 25.1). *D. tuberiformis* first parasitizes its insect host and then continues to garner photosynthates from the plant substrate (White et al., 2002). These photosynthates leak from the intact stylet or wound left by the obliterated scale insect. Either the wound or the close interaction between the fungus and plant substrate may have allowed the jump to occur. This host shift would have provided a broad range of new possible hosts to associate with and would explain the proliferation of plant parasitic clavicipitaleans (i.e., >50 species) within a single clade (clade D, Figure 25.1).

The evolution of plant association through scale insect parasitism may also be occurring within the lineage in clade B (Figure 25.1). In clade B there are strict entomopathogens such as *Cordyceps militaris* (Figure 25.4), *C. pruinosa*, and some *Lecanicillium* anamorphs. However, also in this clade are scale insect pathogens (e.g., genera *Hyperdermium*, *Ascopolyporus*, *Torrubiella*) that, like *D. tuberiformis*, consume their

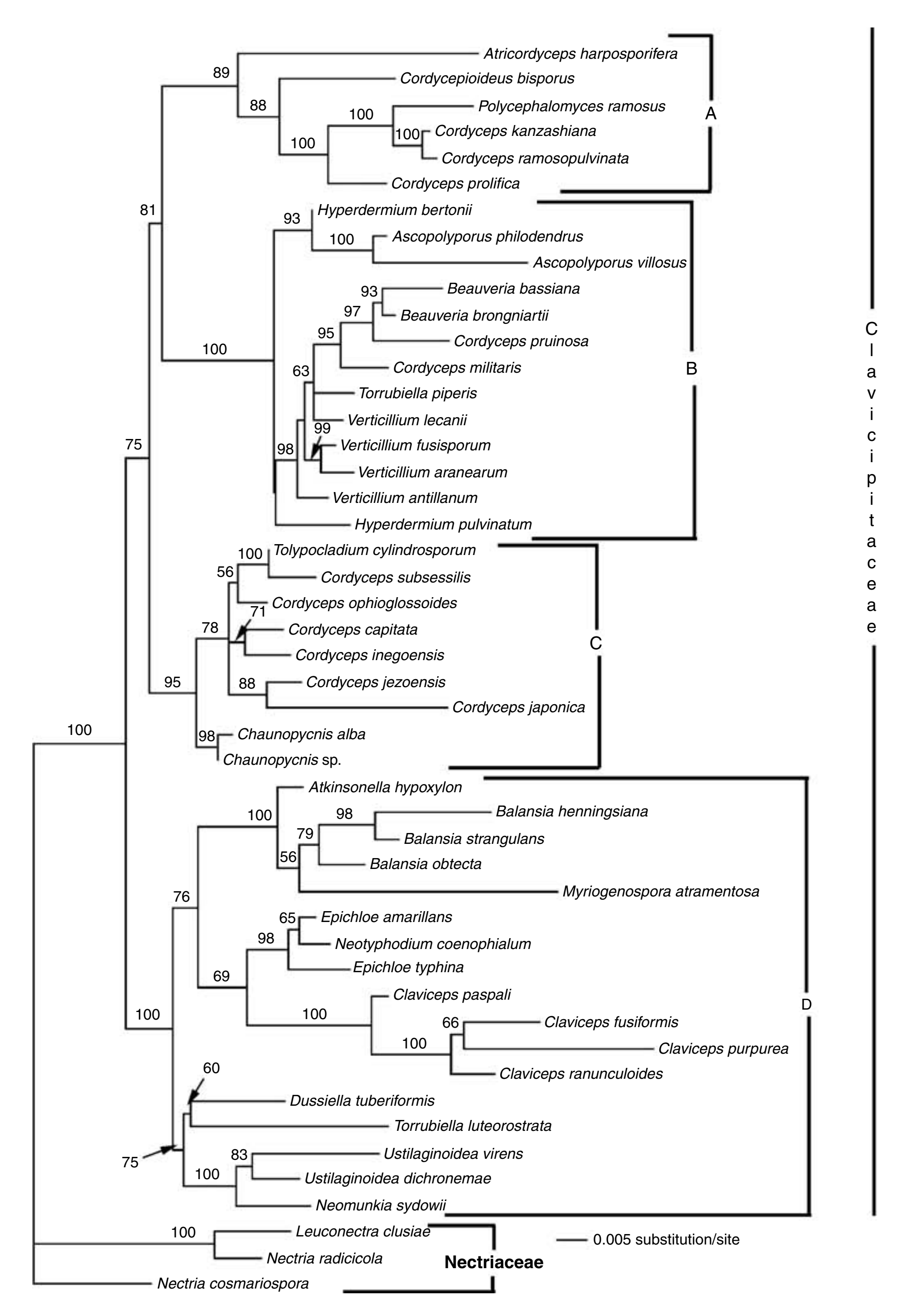


Figure 25.1 Phylogenetic tree (–ln likelihood = 4786.12222) of clavicipitalean taxa and the outgroup Nectriaceae. Major clavicipitalean clades are labeled A through D. This tree is the result of a maximum likelihood analysis using a general time-reversible model, including the percentage of invariable sites and gamma distribution (GTR + I + G; Rodríguez et al., 1990). Taxa were added randomly in 30 replicates with a random starting seed. One tree was held at each step during stepwise addition using the TBR algorithm. MrBayes 3.0, a Bayesian phylogenetic inference program (Huelsenbeck and Ronquist, 2001), was used to determine branch support (posterior probabilities). The posterior probabilities are reported on the branch before the node they support and are based on 96,542 trees.

insect host and are thought to continue to get nutrients from their plant substrate (Sullivan et al., 2000; Bischoff and White, 2004). *Beauveria bassiana*, the most derived member of the clade, has shown some ability to live endophytically in plants and kill insect herbivores that feed on the plant host (Bing and Lewis, 1992). Of the four clavicipitalean clades represented in Figure 25.1, the only two clades that exhibit plant endophytism are also the only two clades to include scale insect pathogens. The fungus–scale insect–plant interactions may provide the mechanism for these interkingdom host shifts to occur. It may be that clade B represents an earlier stage in the host shift than what is seen in members of clade D.

25.4 DEFENSIVE MUTUALISMS

The relationships between some clavicipitalean fungi and their hosts are considered to be defensive mutualisms, where plants provide fungi with photosynthates and other nutrients and fungi defend plants from herbivory (Clay, 1988; Schardl et al., 1997). Clavicipitalean grass endophytes of genus *Neotyphodium* (anamorph of *Epichloë*) produce alkaloids that deter herbivores from consuming plant hosts (Yue et al., 2000). An endophyte in sleepygrass (*Achnatherum robustum*) was shown to produce lysergic acid amide, which has the effect of causing animals that consume the plants to sleep for several days and thereafter to avoid consumption of endophyte-infected plants (Petroski et al., 1992). Many insect herbivores have also been shown to avoid feeding on clavicipitalean-infected plants (Clay, 1988). *Epichloë/Neotyphodium* endophytes have been shown to have significant impacts on populations of hosts due to feeding-deterrent effects. Knoch et al. (1993) demonstrated that clavicipitalean endophytes in tall fescue (*Festuca arundinaceae*) altered foraging of seed-harvesting ants. It was also found that leaf-cutting ants in communities containing populations of the grass *Bromus setifolius* showed a shift toward grass populations dominated by endophyte-infected individuals presumably due to selection by the ants or other herbivores (White et al., 2001). Due to defensive mutualism, claviciptalean endophytes likely play a significant role in evolution of some host populations.

25.5 EVOLUTION OF DEFENSIVE MUTUALISM

As briefly discussed above, the early stages of defensive mutualism with host plants may be seen in the scale insect–infecting fungi deeply rooted in clades B and D (Figure 25.1). Species of genera *Hypocrella* (Figure 25.2A), *Hyperdermium*, *Ascopolyporus*, *Torrubiella* (Figure 25.2B), and *Dussiella* infect scale insects that are themselves parasites of host plants. The cost of this protection to the plant substrate may be nutritional sustenance. Epiphytic stromata bear conidia and later perithecia that provide the source of inoculum that protect plants from additional scale insect parasitism. It seems reasonable that the cost of supporting the epibiotic clavicipitaleans is less than the cost of unchecked growth of scale insect populations.

The scale insect host defense exhibited is based on the direct consumption of the insect plant predator by the fungal epibiont. Defensive mutualism in the grass endophytes (e.g., genera *Balansia*, *Epichloë*, *Neotyphodium*) of clade D (Figure 25.1) is based on chemical metabolites produced by the fungus. The endophytes of clade D may have been preadapted for this defensive strategy because of the anti-insect defensive role of their evolutionary precursors. They may also have been preadapted for the chemical defensive strategy by ancestral use of toxic alkaloids to defend the epiphytic fungal stromata. Deeply

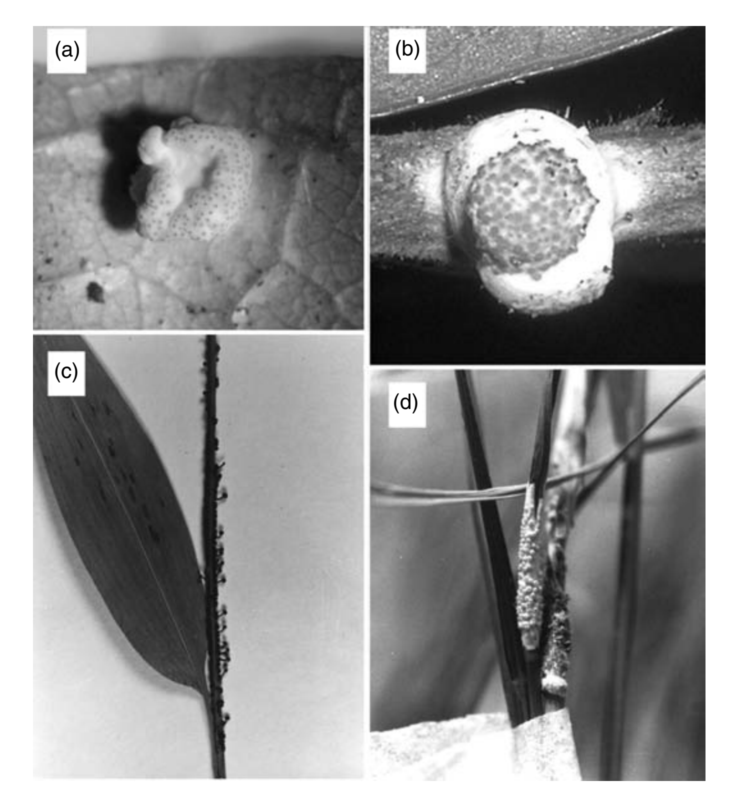


Figure 25.2 Four clavicipitalean taxa, of which two are scale insect pathogens (a and b) and two are biotrophs of monocots (c and d). (a) *Hypocrella* sp. on the underside of a dictomous leaf. (b) *Torrubiella piperis* on the stem of a *Piper* species. (c) *Balansia asclerotica* on a monocot stem. (d) *Epichloë* sp. on the culm of a Poaceae.

rooted in clade D is *Dussiella tuberiformis*. Ancestors similar to *D. tuberiformis* may have been the precursors to the grass endophytic genera. *D. tuberiformis* infects the scale insect, degrades it, and develops a stroma on the surface of the bamboo substrate (*Arundinaria tecta*) using nutrients that emerge through the scale insect's stylet. We have found that stromata of *D. tuberiformis* are a rich source of ergot alkaloids (Koroch et al., 2004). It seems likely that these alkaloids may play a role in defending the long-lived epiphytic stroma from herbivory of birds, insects, and other potential consumers of the fungus. In fact, some insects (e.g., lepidopterans, homopterans) may mimic clavicipitalean parasitism to deter would-be insectivores (Bischoff, personal observation; Figure 25.3). It seems reasonable that such a self-defense strategy could have been shifted to defense of the host plant once a systemic endophytic habit was acquired.

25.6 DIVERSITY OF STROMAL MORPHOLOGY IN CLAVICIPITACEAE

Particular characteristics are usually attributed to members of Clavicipitaceae (e.g., brightly colored stromata; perithecial, cylindrical asci; multiseptate filiform ascospores). However, a great deal of morphological diversity is evident in the family. Typical characters that are easily observable with the naked eye are among the most varied and often the most

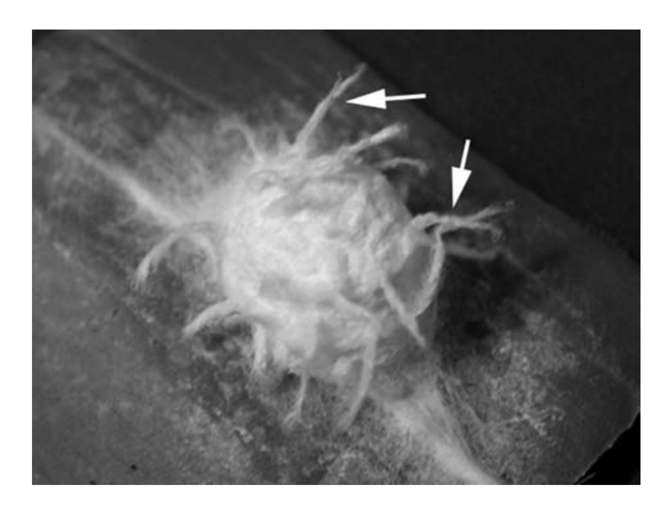


Figure 25.3 Clavicipitalean mimic. Homopteran with carbohydrate extract that resembles *Akanthomyces* synnemata (arrows).

homoplastic. Some of the fungi are stipitate with a perithecial stroma at the apex, or multibranched with multiple perithecial stromata. Closely related species may develop sessile stromata or may even lack a stroma entirely. This kind of morphological variety can be seen among members of clades A and B (Figure 25.1). Species of *Hyperdermium*, *Ascopolyporus*, and *Torrubiella* (Figure 25.2B) produce sessile pulvinate stromata (Sullivan et al., 2000; Bischoff and White, 2003). *Cordyceps pruinosa*, *C. militaris* (Figure 25.4), and all members of clade A produce stipitate perithecial stromata. *Torrubiella confragosa*, a taxon nested within clade B, lacks a stroma altogether and produces its perithecia from a thin film of mycelium covering the host insect's corpse. The use of stromal shape characters has been problematic in clavicipitalean systematics. Traditionally, the presence of a stipe developing from a parasitized insect or truffle along with other typical clavicipitalean characteristics required that the fungus belong to genus *Cordyceps*. The lack of a stipitate perithecial stromata would require placement in another genus that would be selected based on other typically stromatal characteristics. This approach has clearly led to paraphyly, specifically among the insect and fungal associates of Clavicipitaceae. Here many separate lineages of clavicipitaleans were classified in genus *Cordyceps* due to superficial resemblance based on possession of stipitate perithecial stromata.

Other macroscopic characters have been equally misleading. Among members of clade B the placement of perithecia in relation to the stroma (i.e., immersed or exposed) varies without any identifiable regard to phylogenetic relationships. *Ascopolyporus* species, *Hyperdermium bertonii*, *Cordyceps* species, and *Torrubiella piperis* all produce immersed perithecia, while *Hyperdermium pulvinatum* and *Torrubiella confragosa* have perithecia that are mostly exposed (Mains, 1949; Sullivan et al., 2000; Bischoff and White, 2003).

Stromal color also varies greatly in Clavicipitaceae, but does appear to provide some indication of evolutionary relationships. Subclades in clade D (Figure 25.1) are usually associated with a particular range of stromal pigments. Stromata of *Epichloë* are associated with a yellow to white pigment, and *Claviceps* is usually yellow to orange. In addition, the fungal and insect pathogens of clade C have a dark pigmentation, olive to dark brown. However, exceptions to the pigmentation trends are easy to find. The greatest variety occurs in clade B. All members of this clade are brightly pigmented. *Hyperdermium bertonii*,

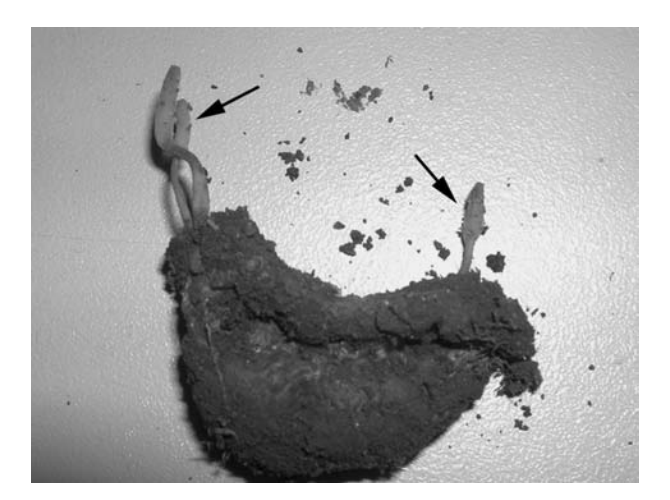


Figure 25.4 *Cordyceps militaris.* Parasitized lepidopteran pupae with emerging *C. militaris* clavae (arrows).

Ascopolyporus philodendrus, and *A. villosus* are orange, purple, and white, respectively. A great deal of color variation is found in the rest of clade B also.

25.7 PHYLOGENY AND TAXONOMY

Pleomorphism is an obstacle to elucidating the full depth of fungal evolution and taxonomy. *Pleomorphism* is defined as the condition of a fungus "having more than one independent form or spore-stage in the life cycle" (Hawksworth et al., 1995, p. 364). While this phenomenon has been recognized in mycology since de Bary (1854) made the first link between an anamorph and teleomorph state, it has continued to hamper systematic and nomenclatural progress. Anamorphic groups have been traditionally placed in the phylogenetically uninformative group Deuteromycota, or Fungi Imperfecti, and have largely maintained a distinct nomenclature from the teleomorphic fungi. Because comparisons between anamorphic and teleomorphic states could not be made based on homologous morphological structures, the separation of the groups based on phenetics was practical. However, this created two taxonomic systems that do not represent the shared evolutionary past of the Eumycota. Molecular data have provided the opportunity to develop phylogenetic hypotheses that include both anamorphic and teleomorphic fungi. The DNA sequence data provide a common arena where homologous characters may be assessed.

Determining a morphological concept of monophyly in Clavicipitaceae has remained elusive. However, previous hypotheses (e.g., Diehl, 1950) regarding anamorphic states and their utility in delimiting monophyletic groups in the family have been supported to a large degree by modern phylogenetic studies. The sections that follow will discuss some of these studies and their results in greater detail.

25.8 CLASSIFICATION SYSTEMS IN THE CLAVICIPITACEAE

Gäumann (1926) included three groups in Clavicipitaceae, which were later interpreted as subfamilies (Oomycetoideae, Clavicipitoideae, Cordycipitoideae) by Diehl (1950). They

were distinguished based on what Diehl described as "three divergent evolutionary trends in the development of the ascostroma" (p. 10). However, recent studies concerning various taxa in Clavicipitaceae do not support the monophyly of these subfamilies.

Diehl (1950) split subfamily Clavicipitoideae into three tribes (Clavicipieae, Balansieae, and Ustilaginoideae) based primarily on the morphology of their conidiogenous cells. Diehl wrote: "It is questionable whether any natural system of classifying the genera in the Clavicipitaceae can be determined by utilizing as criteria only ascostromatic features" (p. 10). Molecular analyses testing Diehl's hypotheses regarding the monophyly of the tribes have supported his use of conidiogenous cell morphology in distinguishing monophyletic clades in Clavicipitoideae (Kuldau et al., 1997).

Tribe Clavicipiteae includes only genus *Claviceps*. Diehl (1950) distinguished *Claviceps* (the teleomorph of *Sphacelia*) from other taxa in the subfamily based on the development of the *Sphacelia* anamorph on the ovary of its grass host. *Sphacelia* is characterized by the production of glutinous microconidia from simple conidiogenous cells densely arranged in a hymenium-like layer. Phylogenetic analyses have supported the monophyly of this tribe (Figure 25.1). Recent work by Pazoutová (2003) included the morphologically distinct, monotypic genus *Neoclaviceps* within *Claviceps*. However, *Neoclaviceps monostipa* produces septate ephelidial conidia (see *Ephelis* below) similar to those found in *Myriogenospora linearis* (Sullivan et al., 2001). It is still unclear whether this morphological feature represents a homologous character between these two taxa or is a product of convergent evolution. A more detailed study of *Neoclaviceps monostipa* and *Myriogenospora linearis* is required to help elucidate their relationships.

Epichloë, *Atkinsonella*, and *Myriogenospora* develop microconidial states similar to *Sphacelia* but were segregated from tribe Clavicipiteae based in part on the stromatal development of these taxa on the host grass inflorescence and leaf blade. Furthermore, the microconidia of *Atkinsonella* and *Epichloë* develop on the stromal surface and are dry, not glutinous. Like *Balansia*, ephelidial macroconidia referable to the form genus *Ephelis* are produced by *Atkinsonella* and *Myriogenospora*. These ephelidial conidia develop holoblastically from simple conidiophores in a sympodial fashion (Rykard et al., 1984). Diehl (1950) interpreted these conidial states as a transition from microconidia along the stromal surface as found in *Epichloë* to the synanamorphs of *Atkinsonella* and *Myriogenospora*, and then the ephelidial conidia of *Balansia*. Thus, Diehl included *Epichloë*, *Atkinsonella*, *Balansia*, and *Myriogenospora* in tribe Balansieae. More recent phylogenetic analyses using molecular data do not support the monophyly of Balansieae. However, these analyses support the monophyly of taxa that produce the *Ephelis* anamorph to the exclusion of *Epichloë* (Glenn et al., 1996; Kuldau et al., 1997; Bischoff et al., 2004). *Epichloë* is also monophyletic with a distinct anamorph now referred to as *Neotyphodium* (Glenn et al., 1996). The results of Glenn et al. and others suggest that Diehl may have misinterpreted the synanamorphs as a transitional state in the group's evolution.

Diehl (1950) defined the tribe Ustilaginoideae to contain graminicolous clavicipitaleans that produce subglobose conidia from pores along the surface of hyphal-like conidiophores (Figure 25.5). Ustilaginoideae includes the anamorphic genera *Ustilaginoidea*, *Munkia*, and *Neomunkia*. Both *Neomunkia* and *Munkia* develop subglobose stromata that are erumpent from the culms of their bamboo hosts (*Chusquea* sp.). Like *Claviceps*, *Ustilaginoidea* spp. infect the florets of various subtropical and tropical grasses and replace the seed with a smut-like ball. *Ustilaginoidea virens*, the most studied species of the tribe, is the causal agent of False Smut of rice (*Oryza sativa*) and can be found wherever rice is grown (Ou, 1972).

Members of Ustilaginoideae have never been connected to a teleomorph with any certainty. However, Hashioka et al. (1951) described sclerotia with stipitate perithecial

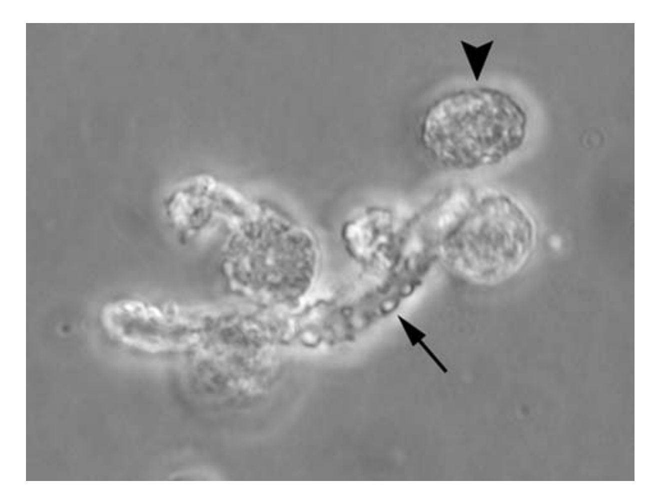


Figure 25.5 *Ustilaginoidea virens*. Smut-like conidia (arrowhead) that develop from raised pores (arrow) along the hypha-like conidiophore.

stromata developing from *Ustilaginoidea virens* smut balls. Despite the differences in anamorphic morphology between the two genera, he named the organism *Claviceps oryza-sativa*. von Höhnel (1911) believed that *Munkia* was connected to the rare clavicipitalean teleomorphic genus *Mycomalus*. Diehl (1950) included *Mycomalus* in subfamily Oomycetoideae.

Recent phylogenetic studies conducted regarding tribe Ustilaginoideae found the group to be monophyletic and distinct from *Claviceps* (Bischoff et al., 2004). In addition, analyses strongly supported the inclusion of Ustilaginoideae in subfamily Clavicipitoideae (clade D) as predicted by Diehl (1950; Figure 25.1).

25.9 CONIDIAL STATES AS INDICATORS OF EVOLUTIONARY RELATIONSHIPS

While morphological traits, such as stromal color and conidial state, were shown to be somewhat informative in determining monophyletic groups in Clavicipitoideae, we were interested in determining the utility of anamorphic or conidial state morphology in delimiting monophyletic groups in other taxa, particularly the insect pathogens of Clavicipitaceae. Determining phylogenetically informative morphological characters is of particular interest due to the homoplasticity of characters previously used in delimiting genera (e.g., the presence or absence of a stipitate perithecial stroma) and the species richness of these groups. In his key to the taxa of *Cordyceps* and *Torrubiella*, Kobayasi (1982) included over 300 entomopathogens from these two genera alone.

Seifert (1985) combined the monotypic anamorphic genus *Blistum* into *Polycephalomyces*. He determined that the ornamented cells found along the stipe of *Blistum tomentosum* and variably shaped conidia were not sufficient to distinguish the species from form genus *Polycephalomyces*. Species of *Polycephalomyces* are synnematous with yellow, ovoid conidia (alpha conidia) produced in a glutinous matrix at the apex (Figure 25.6). In addition, *P. ramosus* was described to produce fusiform conidia (beta conidia) in dry chains along the synnematous stipe as well as alpha conidia at the apex. *Polycephalomyces* spp. are found associated with arthropods and entomopathogenic fungi in genus *Cordyceps*.

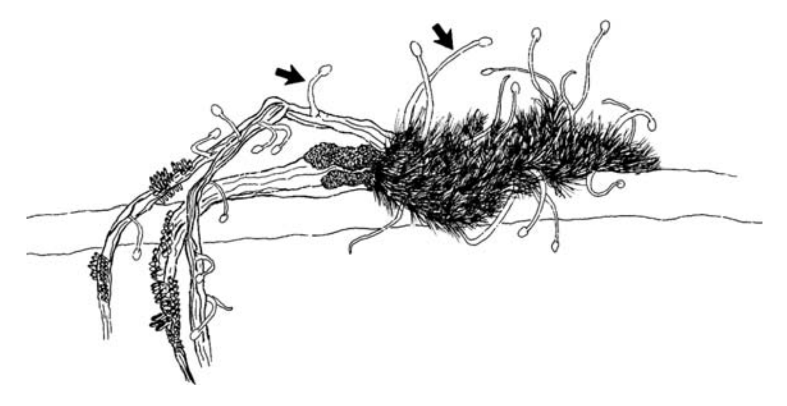


Figure 25.6 *Polycephalomyces ramosus* synnemata (arrows) developing from *Cordyceps* sp. clavae (arrow) and lepidopteran larva. (Drawn by Rachna Patel.)

Thus, it has been unclear whether *Polycephalomyces* is composed of entomopathogens linked to *Cordyceps* or mycoparasites of the latter (Seifert, 1985). *Blistum tomentosum* is a myxomycete pathogen that has been connected to the myxomyceticolous monotypic fungus *Berkelella stilbigera* in Clavicipitaceae (Rossman et al., 1999).

Based on morphological analyses of *Polycephalomyces sensu lato*, Bischoff et al. (2003) determined that *Polycephalomyces formosus* and *P. ramosus* were congeneric, but that *B. tomentosum* (as *P. tomentosus*) was sufficiently distinct to warrant its segregation from *Polycephalomyces*. Their phylogenetic analysis based on molecular data supported these results as well as the inclusion of all three taxa in Clavicipitaceae. However, *B. tomentosum* was not resolved within any distinct group of clavicipitaleans. These results suggested that the anamorphic morphology of *Polycephalomyces sensu stricto* was sufficient to determine the monophyly of the group.

We determined that *Cordyceps ramosopulvinata* and *C. kanzashiana*, both entomopathogens, develop a *Polycephalomyces* anamorph (Bischoff et al., unpublished data). Furthermore, phylogenetic analyses in that study included these taxa along with *P. formosus* and *P. ramosus* in a single clade. These results show that fungi with a *Polycephalomyces* anamorph are monophyletic and appear to be entomopathogens.

The polypore-like development of the teleomorphic genus *Ascopolyporus* makes it unique among clavicipitaleans (Möller, 1901), and along with *Hypocrella*, it represents the only taxa of Diehl's subfamily Oomycetoideae to be included in a modern phylogenetic context. The conidia of *Ascopolyporus* are subcylindrical and multiseptate upon maturation (Bischoff and White, in press). The only other genus in Clavicipitaceae to exhibit these conidial features is *Hyperdermium* (Sullivan et al., 2000). Also, like *Hyperdermium*, the conidia of *Ascopolyporus* develop in heads at the tips of simple conidiophores.

A phylogenetic study regarding *Ascopolyporus* supported the close relationship of this genus to *Hyperdermium* (Figure 25.1). The two species of *Ascopolyporus* included in the analyses were monophyletic, with *Hyperdermium bertonii*, the type species of the genus, placed directly basal to them.

Difficulties in using the anamorphic state in determining monophyly in the family arise when considering the anamorphic genus *Verticillium sensu lato*. The genus was grossly polyphyletic and found to be connected to various orders in Ascomycota (Gams, 1971). Clavicipitaleans with connections to *Verticillium* were included in *Verticillium* sect. *Prostrata* (Gams, 1971). However, recent work based on morphological and molecular data have segregated the section into distinct genera (Gams and Zare, 2001). These taxa

were segregated based on host substrate, cultural characteristics, conidial shape, and phylogenetic placement. Most taxa previously included in *Verticillium* sect. *Prostrata* were placed in the newly erected genus *Lecanicillium*. The type species of *Lecanicillium* is *L. lecanii* and is linked to the scale insect teleomorph *Torrubiella confragosa*. The type species of *Cordyceps*, *C. militaris*, is a pathogen of lepidopteran pupae and also produces a *Lecanicillium* anamorph.

A new scale insect pathogen was collected in a lowland wet forest in Panama in the summer of 2003. Its teleomorphic features placed it in genus *Torrubiella*. However, genus *Torrubiella* is polyphyletic and provides little information regarding its phylogenetic placement within Clavicipitaceae. This new species, *T. piperis*, develops a *Verticillium*-like state on the stromal surface (Bischoff and White, 2004). Its color, conidial shape and development, and cultural characteristics were in agreement with taxa of *Lecanicillium*. Phylogenetic analyses using rDNA large subunit (LSU) support the placement of *T. piperae* among other *Lecanicillium* taxa (Figure 25.1) and closest to *T. confragosa*.

The results of the studies by Bischoff and White (2004) and Gams and Zare (2001) do not support the monophyly of *Lecanicillium*. The genus appears to be distinct to a particular clade of entomopathogenic clavicipitaleans, but *Beauveria bassiana* and *B. brongniartii* with polyblastic conidiogenesis are the most derived taxa in the clade with *Lecanicillium* (clade B, Figure 25.1). Therefore, taxa with the *Lecanicillium* anamorph are unique to a particular portion of Clavicipitaceae but cannot be considered monophyletic.

As Diehl (1950) predicted, the utility of anamorphic characters goes beyond Clavicipitoideae. This appears to be especially true for the more morphologically complex anamorphic states (e.g., *Polycephalomyces*, *Beauveria*, *Aschersonia*). Morphologically simplistic anamorphs such as *Verticillium*-like and *Acremonium*-like states occur in multiple groups in Clavicipitaceae. The morphological similarities that these groups share are likely the product of convergence and have evolved multiple times as exemplified by the research conducted regarding the taxa of *Verticillium sensu lato* (Zare et al., 2000). Thus, anamorphic characters alone are not sufficient to delimit clavicipitalean taxa into monophyletic groupings.

Unfortunately, it seems that there is no magic bullet that will allow us to delimit clavicipitaleans into monophyletic groups. While anamorphic morphology is a powerful tool in delimiting groups, a more total evidence approach including host substrate and stromal characteristics needs to be implemented if we are to make order out of the disorder in Clavicipitaceae.

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26

Ecological Fitness Factors for Fungi within the Balansieae and Clavicipiteae

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26.1 INTRODUCTION

The Gramineae are defined as monocotyledonous plants that have hollow, or rarely solid, jointed stems and leaves in two rows on stems, with embryos lateral to the endosperm and seeds that are borne between two scales. Such a simple definition characterizes one of the largest and most important families of flowering plants whose origin dates back to the Jurassic period of the Mesozoic era, approximately 130 million years ago (Thomasson, 1986). The uncertainty is due to a lack of a definite grass archetype in the fossil records. Members of the approximately 8000 species occur from forests, forest margins, and cool-temperate open meadows, to open savannas and semiarid tropic and subtropical regions. This present-day distribution is a consequence of several evolutionary lines leading to a diversity of species.

The grasses are considered to have originated as understory plants in forest, and from this origin the family developed key characteristics that allowed species to reach the climax association in open habitats (Stebbins, 1981). It is in this habitat that the greatest diversity of species is found, which is due in part to the symbiotic relationship with grazing animals, especially ruminants, indicating that these species have the most evolved traits. Other components in grass evolution that allowed for exploration of the open habitat are the developments of the perennial habit, unique intercalary meristem, and the absence of

poisonous secondary metabolites. While the association of grasses with animals might have favored the distribution of species, the downside of this association is herbivory and the resulting grazing stresses compounded by the semiarid environment. Once the exploration of the open habitat was achieved, pressures apparently developed against the quasi-association of animals with grasses. Grasses lack poisonous secondary metabolites that assist other plants in their long-term strategies to compete against herbivory. Fungi are notorious for their production of poisonous secondary compounds, which can serve important functions to grasses — thus the evolutionary drive for the association of fungi with grasses. In addition to grazing animals, fungi became associated with grasses, possibly pathogenic at first, but in time fungi became biologically associated with grasses, and many of these developed into mutualistic symbioses.

The fungi of concern belong to a relatively small grouping of species within the Clavicipitaceae (Figure 26.1; Lewis et al., 2003). The family was initially placed in the order Hypocreales, although earlier reexamination of this placement was met with some controversy. However, due to molecular technology, the original relationship of this group among the Hypocreales was supported (Spatafora and Blackwell, 1993; Glenn, 1995; Kuldau et al., 1997). This group of fungi shares a common feature in being systemically associated with grasses, sedges, and rushes as biotrophic parasites. However, there are



Figure 26.1 A partial relationship of the Balansieae and Clavicipiteae within the family, according to Lewis et al. (2003), showing clear separation of the genera from each other; data were based on a maximum likelihood tree using the 6ST-GTR + G + 1 evolutionary model (with the author's permission). Note that according to this model a fourth genus, *Atkinsonella*, was not retained as a distinct genus and was placed in the genus *Balansia* (Lewis et al., 2003).

effects; these include low to no seed production, and depending on the fungal species, infected hosts are dwarfed or fasciated (Diehl, 1950; Luttrell and Bacon, 1977). The manner by which nutrients are obtained within symbiotic associations varies; some fungi develop into hemibiotrophs, while others are complete biotrophs. The morphological association of the fungi with grasses occurs as either epicuticular (*Myriogenospora*), epibiontic (*Claviceps*), or endophytic (*Epichloe*, *Neotyphodium*, and *Balansia*). Research into the nature of the associations of this group of fungi with grasses is important because grasses are the most important group of plants to humankind.

The species of the Balansieae and Clavicipiteae are obligate biotrophs, although in some species, such as the Clavicipiteae, the biotrophic stage is fleeting and develops into a hemibiotrophic stage. The following host-associated characteristics appear to be hallmarks of these biotrophic fungi: (1) lack of highly developed infection structures, (2) compatible association that may be long term and intercellular, and (3) nutrient exchange occurring along the hyphae of the biotrophic plant–fungus interfaces, although in *Claviceps* a foot-like organ is produced that serves the purpose of drawing nutrients into the developing sclerotium (Luttrell, 1977, 1980, 1981; Bacon and Luttrell, 1982; Mey et al., 2002).

The association of these two groups of fungi with grasses results in the accumulation of several classes of fungal-specific compounds; in the Balansieae the accumulation is in the foliage, and in the Clavicipiteae certain classes of compounds accumulate in sclerotium. Historically, these metabolites have been described as totally defensive in their action with other organisms. However, these metabolites may have other roles that may be both physiologically and ecologically relevant within the association. The absence of toxic compounds and other secondary compounds in the grasses is considered in evolution the basis for the establishment of a compatible association with the fungi. In this chapter, the apparent inter- and intraspecific competition and ecological outcome of establishment of fungal biotrophy are discussed, which offers further discussions on competition within symptomless associations, exhibited by the Balansiae, and biotrophic competition within the disease state, exhibited by the Clavicipiteae.

26.2 HOSTS ASSOCIATIONS AND EVOLUTION

The Balansieae and Clavicipiteae are distributed among the six subfamilies and several tribes of grasses, especially those that are considered the more highly evolved, e.g., the Panicoideae (Table 26.1). Most species of grasses live within an open environment that may be wet to humid to arid, occupying grassland areas from the tropics, subtropics, and temperate zones. The Panicoideae is a subfamily that is characterized with a diversity of species displaying a range of variation in both photosynthetic pathway (C_3 and C_4) and inflorescence structure. Species of this subfamily are climax species within grasslands of the tropics and other regions of the world, and this subfamily contains the largest number of grass species. It is interesting that the Panicoideae serves as host for the largest number of species of both clavicipitalean species (Table 26.1) and the seven tribes in this subfamily; only two are listed as host for the clavicipitalean fungi. In the Balansieae, 9 of the 10 known species of *Epichloe* are associated with Panicoideae; the only exception is the Bambusoideae that is parasitized by *E. brachelytri.* A similar distribution is observed for *Myriogenospora* species: one species is associated with one host species of the Bambusoideae, and the other is associated with seven grass species of the Panicoideae. Nevertheless, the distribution of these fungi on these grasses is widespread and follows the geographic distributions of the host, which occur from the subtropical to temperate regions of the world. The distinction of the clavicipitalean fungi may be divided along geographic

Table 26.1 Distribution of the World's Species of *Claviceps*, *Balansia*, *Epichloe*, and *Myriogenospora* among the Tribes of Grasses[a]

Subfamily[b]	**Tribe**[c]	**Number of *Claviceps* Species**	**Number of *Balansia/Epichloe* Species**[d]
Bambusoideae	Bambuseae, Oryzeae	1	5
Centothecoideae	Centotheceae	1	3
Arundinoideae	Arundineae, Aristideae	0	2
Pooideae	Stipeae, Poeae, Bromeae, Triticeae, Aveneae	1	1
Chloridoideae	Eragrostideae, Cynodonteae	6	2
Panicoideae	Paniceae, Andropogoneae	30	22

[a] *Claviceps* species summarized according to Alderman (2003); *Balansia/Epichloe* species summarized according to Lewis et al. (2003) and Leuchtmann (2004). Grass tribes are based on the 6 subfamilies and 39 tribes delineated according to Clayton and Renvoize (1986).

[b] Subfamilies are presented from the most primitive, Bambusoideae, up to the most advanced, Panicoideae. Excluded are the nongrass species, Cyperaceae, although they are infected by clavicipitalean species (three additional *Claviceps* species and two *Balansia* species).

[c] Listed are those tribes reported to serve as host; others are omitted.

[d] Numbers consist of sexual and asexual endophytes (*Ephelis* and *Neotyphodium,* respectively) and represent a combined total of all *Balansia*, *Epichloe*, and *Myriogenospora* species.

boundaries. Thus, we have temperate and tropical–subtropical species of both Balansieae and Clavicipiteae.

These two groups of fungi are of tropical origin (Langdon, 1954; Diehl, 1950), and accumulated data suggest that the temperate species are more specialized, i.e., highly evolved, than the tropical species (Brady, 1962; Didek-Brumec et al., 1996; Jungehülsing and Tudzynski, 1997; Pazoutová, 2001; Alderman, 2003). There is no evidence for coevolutionary relationships between the clavicipitalean fungi and their hosts. In a more extensive study it was concluded that in the case of the *Claviceps* species, evolution did not directly follow the scheme of grass evolution (Mathews and Sharrock, 1996), and we suspect that evolution in the Balansieae also did not follow grass evolution. Indeed, the data suggest that infection of grasses at a given habitat depended on the availability of the many species and diversity within the subfamily Panicoideae and on climate changes, especially those occurring in the Oligocene and late Pliocene and Pleistocene periods (Raven and Axelrod, 1974; Mathews and Sharrock, 1996). Migration of these fungi followed spreading of the Panicoideae into the tropical semiarid and temperate regions of the world. The present-day distribution is probably confounded by dissemination by humankind and modern agricultural practices. There are other interesting hypotheses favoring coevolution of clavicipitalean fungi with grasses (Schardl et al., 1997; Lane and Christensen, 2000). However, very little information was presented on the grasses parasitized by the fungi, and their place within the evolutionary events was anticipated and discussed as occurring with the clavicipitalean fungi.

26.3 COMPETITION FOR AND WITHIN THE ENDOPHYTIC NICHE: THE BALANSIEAE

Antagonisms among microorganisms are strategies that maintain both inter- and intraspecific competition, which is particularly important among those organisms that are ecological homologues, such as microorganisms that colonize the intercellular spaces of plants as endophytes (Stone et al., 2000). Endophytic organisms in general are not host specific, and some plants, presumably because of a general compatible mechanism, can serve as hosts for more than one species, although not necessarily at the same time. Most of the fungi are compatible with plant hosts, which is a salient feature of biotrophic fungi.

Ecological homologues do not occupy the same niche. Within any given grass population species of the two clavicipitalean groups may be parasitic on the same host, but never will there be two species coexisting within the same endophytic niche on the same grass. Why are there not more than one species found within the same host? The basis for this may in part be due to the very nature of compatibility factors, which might be physiologically expressed as biotypes. However, there are alternatives for these very specific infections. Dominance by a particular endophytic species within the intercellular spaces of grasses is due to an exclusionary principle, which is assumed to rely upon either antagonism, nutrient competition, or systemic-induced resistance as a means of maintaining dominance within the intercellular habit. In the instance of phyllosphere bacteria, it has been established that it is competition for limiting resources, not antibiosis, that is the primary mechanism of antagonism (Lindow, 1987; Wilson and Lindow, 1994; McCully, 2001). Studies demonstrating that only one species occupies intercellular spaces of an individual grass have not been determined. Thus, if ecological homologues compete, dominance would depend on the competition for nutrients and the vigor of the endophytic species, which is correlated with the ability for carbon source utilization (Cheplick et al., 1989; Mey et al., 2002). According to Richardson (2000), the carbohydrates found in the apoplasm include glucose, fructose, and mannitol, which were present in significantly higher concentrations in endophyte-infected *Poa ampla* than in noninfected grass. Competition for infection of the endophytic niche in *P. ampla* by another endophytic species would occur only if that species could utilize all sugars (and other compounds) at a frequency greater than the others.

Coexistence within the endophytic niche is also under the influence of biotic and abiotic factors, only a few of which have been determined. Interactions between endophytic fungal species and nonendophytes indicate that niche overlap between competing species depends on environmental factors such as water (Marín et al., 1998). We do know, however, that from one tiller only one species is isolated, while within the population of grasses, other endophytic species can be isolated from this same host species (Cheplick et al., 1989; An et al., 1993; Bayman et al., 1998; Wille et al., 1999). The concept of noncoexistence by ecological homologues was clearly established by observations of double infections of *Andropogon virginicus* by both *Balansia henningsiana* and *Myriogenospora atrementosa*. In this instance the infection of *B. henningsinana* is endophytic, while that of *M. atrementosa* is superficial, infecting the tips of a leaf and inflorescence from the same plant (Luttrell and Bacon, 1977). Such double-infected plants are rare, but they do occur within limited locations rather routinely, suggesting either environmental interactions or biotrophic competitive interactions.

26.4 BIOTROPHIC AND HEMIBIOTROPHIC INTERACTIONS: THE CLAVICIPITEAE

All species of *Claviceps* have an initial biotrophic growth phase before becoming hemibiotrophs. During this stage, a foot-like structure is developed that is associated with the host's vascular system (Mower and Handcock, 1975; Luttrell, 1977, 1980; Didek-Brumec et al., 1996; Shaw and Mantle, 1998; Mey et al., 2002; Alderman, 2003). While there is a reduction in host seed, usually the plant remains healthy. However, in the case of *C. sorghii* or *C. Africana* there is such a large amount of inoculum due to the infection of all male sorghum cultivars. This may be caused by the physiology of these agricultural cultivars, resulting in death of plants (Frederickson et al., 1991; Bandyopadhyay et al., 1998). Information on the behavior of wild-type sorghum has not been reported.

The topographical signal that induces the change from hemibiotrophy to necrotrophy is unknown. However, the switch is coordinated and usually results in the death of cells. The stage of infection (adhesion to the stigma, growth into the style, and penetration into host tissue) is endophytic and intercellular, but during the later stages a few hyphae become intracellular. The time for this lasts for a few hours or a week, depending on the species and conditions during infection. During this period the host shows no signs of infection. Thus, the symptomless state of the Clavicipiteae is relatively transient, while that in most of the Balansieae it intransient. This is especially true for the *Epichloe* species. Nevertheless, there is ample evidence that the Clavicipiteae and Balansieae manipulate the host's physiology. What metabolites does the fungus produce or host contribute to the outcome of the brief interaction? The secondary metabolites discussed below, as well as others, might regulate the interaction, resulting in a shift from a symptomless association, which we presume is less competitive with the host, to one that is highly competitive and develops to a disease state.

26.5 BIODEFENSIVE COMPOUNDS

The list of substances with known biological activity that are produced by clavicipitalean fungi is extensive, but practically all are alkaloids and are related to various aspects of tryptophan metabolism, which is directly related to the synthesis of ergot alkaloids (Berde and Schild, 1978; Tsai et al., 1995; Kren et al., 1997a, 1997b), an important product of this association. Tryptophan metabolism apparently is central to the success of clavicipitalean fungi at the physiological and ecological levels. Ergot alkaloids have several pharmacological properties, most of which are valued in human medicine, resulting in hundreds of studies on the mechanism of action from this class of compounds. In most instances these studies have human relevance, and all suggest some role in the long-term ecological success of producing organisms. However, acute toxicity, expressed by most ergot alkaloids, is difficult to rationalize at the ecological level. There is some information to suggest that ergot alkaloids and other tryptophan metabolites have a role in the physiological interaction by altering plant cell membrane, increasing the flow of sugars beyond that normally expected from the simple flow from a source-and-sink mechanism (Lepp and Peel, 1971; Rehacek, 1991; Scigelová et al., 1995; Didek-Brumec et al., 1996). Very little is known about the biological activity of other alkaloids produced within the association of the two tribes. Below is a brief review of relevant compounds whose biological activity is known for specific toxicological or plant physiological and ecological responses. More detailed studies on structures and classical interpretations of toxicological activity should be sought in more comprehensive reviews (Bove, 1970; Berde and Schild, 1978; Groger,

1978; Flieger et al., 1984; Porter, 1994, 1995; Adcock et al., 1997; Kren et al., 1997a; De Groot et al., 1998; Isaka and Kittakoop, 2001).

26.5.1 Simple Indoles and Auxins

Several simple indole alkaloids have been isolated from cultures of endophytic fungi; these include the indole glycerols (3-indolybutanetriol and 4-(3)-indolecarboxylate) and the simple auxins (3-indoleacetic acid, 3-indoleacetamide, 3-indole ethanol, and methyl-3-indolecarboxylate) (De Battista et al., 1990; Porter, 1994, 1995) These substances are isolated from all cultures of the *Balansia* spp. and some isolates of *Neotyphodium coenophialum*. While the effect of the production of the simple auxins' indoleacetic is obvious on plants, the effects of the other simple indoles on the symbiosis are unknown, but may have an ecological function related to perception of seasonal changes by small herbivores relative to reproduction efficiency, resulting in diminished grazing of the infected (Porter, 1994). Thus, this group of simple indoles might serve as the key signal that provides control of small herbivores, directly or indirectly, by controlling the degree of grazing, which will directly affect population densities.

26.5.2 Pyrrolizidine and Pyrrolopyrazine

The activity of these alkaloids has been reviewed (Bush et al., 1982, 1993a, 1993b, 1997; Blankenship et al., 2001) and can be summarized as follows. Although these substances were originally isolated from several other plants, the lolines have recently been shown to be produced by *N. coenophialum* in tall fescue (Bush et al., 1997). Their biological activity on cattle is to decrease feed intake and in small laboratory animals, weight gain is reduced, therefore suggesting that this group is an effective feeding deterrent. They act alone or synergistically with specific groups of toxins to deter insect predation (Siegel et al., 1990).

Peramine, the only pyrrolopyrazine isolated from this group of fungi, is produced within most of the *Neotyphodium/Epichloe*-infected grasses (Rowan, 1993; Prestidge and Ball, 1993; Roylance et al., 1994; Ball et al., 1995). Peramine is one of the few metabolites that apparently has a very specific biological activity: insect feeding deterrent. This metabolite is very active in preventing feeding activity of Argentine stem weevil on perennial ryegrass. While isolated initially from *Neotyphodium lolii* (Rowan, 1993), it is common to most *Neotyphodium/Epichloe* species and has not been looked for in the *Balansia* and *Claviceps* species. Its role in the ecological interaction with insect predators of these grasses offers strong supports for competitive-advantage endophyte-infected grasses. Peramine interacts with the ergot alkaloids to produce more pronounced effects, increasing the spectrum of activity to include aphids (Siegel et al., 1990).

26.5.3 Steroidal and Related Compounds

Several steroidal compounds have been isolated from either *Neotyphodium*-infected grasses or *Claviceps* species. Ergosterol, ergosterol peroxide, ergosta-4,6,8(14),22-tetraene-3-one, and its triene-3-one derivative have been isolated from both *Claviceps* and *Balansia* species (Porter et al., 1975; Davis et al., 1986). Most of these steroids are components of the fungal cell membrane or cell wall complex and are used as indicators for infection, both qualitative and quantitative. Their roles in the long-term survival strategy of clavicipitalean fungi are unknown, and because ergosterol is a common component of the cell membrane of these fungi, the occurrence of the other ergosterol-related metabolites might well represent chemical artifacts of culture or extraction while analyzing for ergosterol.

Antifungal activity has been reported, primarily from stromata of *Epichloe typhina* on timothy. Although not one compound has been positively identified as being responsible for reducing the leaf spot fungus *Cladosporium phlei* on *Phleum pratense*, the metabolites

include sesquiterpene alcohols, furanones, benzopyranone and related metabolites, phenolic glycerides, two sphingolipids, and anthrax–steroids. Most of the substances have related metabolites that are strong fungicides, especially the phenolic glycerides. The sphingolipid mycotoxins that are produced on maize, the fumonisins, by *Fusarium verticillioides* are responsible for several livestock and poultry poisonings, as well as human toxicity and animal carcinogenesis (for review, see Riley et al., 1993, 2001). Thus, the toxicological range for *Epichloe*-infected grass may now extend into the realm of human health and toxicity. Again, examinations for these and related metabolites from other clavicipitalean fungi have not been done.

26.5.4 Indole Diterpenoids

The lolitrems are a product of the *Neotyphodium/Epichloe* complex. These species and several other fungi, including *Claviceps papspali*, also produce the related indole diterpenoids paxilline. These are neurotoxins and apparently are synthesized from paspalline, paxilline, and 13-dooxypaxilline, all of which are found in several clavicipitalean fungi as well as other unrelated fungi (Porter et al., 1977, 1985). Because these metabolites are not necessarily fatal, their action on grazing animals could be considered deterrent, as animals consuming contaminated forages are uncoordinated and no longer graze as extensively as before.

26.5.5 Ergot Alkaloids

Chemically, all ergot alkaloids have the ergoline ring as the common structural feature, which may have various substitutions along the ring. The nature of the ring substitution describes the specific group of ergot alkaloids; there are four basic groups: (1) clavine alkaloids, (2) simple lysergic acid derivatives, (3) peptide alkaloids, and (4) lactam ergot alkaloids. The production of each group of ergot alkaloid is more dependent on habitats, host, and fungus genetics than host-specialized interactions, indicating that there is no variety or chemoraces, as was assumed for years (Pazoutová et al., 2000). However, there are ecological strains, which in the case of *C. purpurea* has been distinguished by random amplified polymorphic DNA (RAPD) typing (Pazoutová et al., 2000).

The clavine alkaloids are considered to be very simple types of ergot alkaloid that are produced by most species of claviceps and are considered precursors leading to the more complicated alkaloids — the lactam ergot alkaloids and peptide alkaloids. *Neotyphodium* and *Claviceps* produce the clavine alkaloids, and most of the *Balansia* species tend to produce only the clavines (Floss, 1976; Berde and Schild, 1978; Groger, 1978; Bacon et al., 1986; Lyons et al., 1986; Arechavaleta et al., 1992; Kren et al., 1997a). Because the clavine group is not as toxic as the others, most *Balansia*-infected grasses are not as toxic to livestock as the ergot alkaloids produced by other clavicipitalean fungi. The clavine alkaloids and their derivatives are by far the most numerous in terms of chemical structures and are also produced by several nonclavicipitalean fungi, such as *Aspergillus fumigatus, Pennicillium roquefortii, Pennicillium islandicum,* and *Pennicillium aurantio-virens.*

The simple lysergic acid derivatives are more biologically active and occur in species of *Claviceps* and *Neotyphodium*. However, most are produced by *C. purpurea* and *C. paspali.* These ergot alkaloids are very active biologically, and grasses or sclerotia containing these are historically recorded as being involved in human toxicity due to the almost select production of apparent races of *Claviceps* species that parasitized rye, barley, and wheat.

The ergopeptines group of ergot alkaloids is produced by species of *N. coenophialum* and *C. purpurea*, typically represented by ergovaline and ergotamine, respectively

(Bacon et al., 1986; Lyons et al., 1986; Powell and Petroski, 1992; Adcock et al., 1997). This group is by far the most exploited pharmacologically and has had an enormous impact on the drug industry. The ergopeptams are similar to the ergopeptines and are equally toxic to mammals. Both groups are considered the most complex of the ergot alkaloids. The ergopeptam alkaloids are only known to occur in *C. purpurea* (Flieger et al., 1984).

26.6 SUMMARY

In general the clavicipitalean fungi are numerous and highly successful antagonists, primarily of the Panicoideae subfamily of grasses, some of which are mutualistic in their association with plants (White and Cole, 1985, 1986; Riesen and Close, 1987; White, 1987; Sieber et al., 1988; Wilson, 1996; Clement et al., 1997, 2001; Rodrigues and Samuels, 1999; Stone et al., 2000; Adams and Kloepper, 2002). The *Neotyphodium* endophytes are known to increase plant growth rate and herbage yield, reduce insect and mammalian predation, and produce tolerances to several abiotic environmental stresses (Clay, 1988; Latch, 1993; Bacon, 1994; Funk et al., 1994; Schardl and Phillips, 1997; Malinowski et al., 1998). These benefits have a physiological basis, although not known for each and every positive benefit. At the evolutionary levels both groups of clavicipitalean fungi are related; therefore, varying degrees of mutualism might have coevolved among both groups. Continued research should establish the nature of any and all mutualistic relations within the family. The finding of similar classes of secondary metabolites in both groups strengthens this hypothesis. In addition to chemical coevolutionary evidence, there is ample evidence to suggest common origins for most clavicipitalean fungi, including molecular and genetic evidence (Scott and Schardl, 1993; Spatafora and Blackwell, 1993; Schardl et al., 1997; Annis and Panaccione, 1998; Schardl, 2001), implying that other similarities within this family might exist. Secondary metabolites might form the basis for most host modifications, and more information of specific aspects can be obtained from reviews on this subject (Siegel et al., 1985; Cheplick and Clay, 1988; Clay, 1990; Schardl and Phillips, 1997).

Finally, because of their unique habit, endophytic microorganisms are exploited as biocontrol agents. Further, endophytes are also exploited for their future uses, based on endophytic delivery mechanisms, i.e., surrogate transformation or paratransgenesis. The information presented here is intended to provide the basis for more detailed discussions based on rigorous research results that will provide the foundation for understanding the unique habit of these biotrophic fungi.

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27

Fungal Communities of Seaweeds

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27.1 INTRODUCTION

Algal-inhabiting fungi are known as algicolous, and they represent a taxonomically diverse group of mutualists, endosymbionts, parasites, pathogens, and saprobes that are of evolutionary, ecological, and commercial interest. In this chapter the nature of algicolous filamentous fungi and their associations will be reviewed and the application of molecular fungal detection techniques discussed, detailing the diversity associated with the canopy of the marine alga *Fucus serratus*.

27.1.1 Algicolous Fungi: Historical Background

The first descriptions of marine algicolous fungi appeared at the end of the 19th century, 30 years after the first report of a marine fungus by Montagne (1856). These early reports of Rostrup (1889), Church (1893), Jones (1898), Reed (1902), Rosenvinge (1906), Cotton (1908), Smith (1908), Lind (1913), and Estee (1913) compiled records of algal infections with fungal descriptions, often obtained from sporadic and incidental collections. Sutherland, in a series of papers from 1915a, b, c to 1916a, b, c, started an extensive survey describing fungi growing on algae from the Solent and Scotland, identifying 14 new species, including *Lulworthia fucicola* and *Didymella fucicola* (Table 27.1).

In the decades following Sutherland's publications few investigations were made until the 1960s and 1970s, when detailed accounts of marine algicolous fungi appeared from Wilson and Knoyle (1961) and Webber (1967), with more extensive studies from

Table 27.1 Species of Parasitic Filamentous Fungi Present in Algae or Algal Canopies

Marine Fungus	Alga Host	Symptom of Disease	Reference
	Ascomycota		
Genus Spathulospora			
S. adelpha Kohlm.	*Ballia* spp.	Malformations	Kohlmeyer, 1973c
S. antarctica Kohlm.	*Ballia* spp.		Kohlmeyer, 1973c
S. calva Kohlm.	*Ballia callitricha*	Malformations	Kohlmeyer, 1973c
S. lanata Kohlm.	*Ballia* spp.		Kohlmeyer, 1973c
S. phycophila Cav. et Johns	*Ballia* spp.	Malformations	Kohlmeyer and Volkmann-Kohlmeyer, 2003
Genus Retrostium			
Retrostium amphiroae Nakagiri et Tad. Ito	*Amphiroa zonata*		Nakagiri and Ito, 1997
Genus Hispidicarpomyces			
Hispidicarpomyces galaxauricol Nakagiri	*Galaxaura falcata*		Nakagiri, 1993
Genus Chadefaudia			
C. balliae Kohlm.	*Ballia callitricha*		Kohlmeyer, 1973a
C. gymnogongri (J. Feld.) Kohlm.	*Curdiea coriacea*, *Gigartina intermedia*, *Gymnogongrus norvegicus*, *Grateloupia filicina*, *Laurencia concina*, *L. flexilis*, *L. heterocladia*, *L. tenera*, *L. pygnaea*, *Microcladia cautein*, *Ptilota australasica*	Discoloration of tissue	Kohlmeyer, 1973b
C. marina G. Feld.	*Palmaria palmate*	Discoloration of tissue	Feldman, 1957

C. polyporolithi (Bonar) Kohlm.	*Polyporolithon conchatum*, *Calliarthron* sp., *Corallina* sp., *P. reclinatum*		Kohlmeyer, 1973a
C. schizymeniae Stegenga et Kemperman	*Schizymenia obovata*	Discoloration of tissue	Stegenga and Kemperman, 1984
Genus Lindra			
L. crassa Kohlmeyer et Volkmann-Kohlmeyer	*Sargassum* sp.	Discoloration of tissue	Tubaki, 1969
L. thalassiae Orpurt, Meyers, Boral et Simms	*Sargassum* sp.		Orpurt et al., 1964
Genus Lulworthia			
L. fucicola Sutherland	*Fucus vesiculosus*		Sutherland, 1916a
L. kniepii Kohlm.	*Lithophyllum*, *Porolithon*, *Pseudolithophylum*	Discoloration of tissue	Kohlmeyer, 1963
Genus Trailia			
T. ascophylli Sutherland	*Ascophyllum nodosum*, *Fucus* sp.		Sutherland, 1915c
Genus Haloguignardia			
H. cystoseirae Kohlm. et Demoulin	*Cystoseira*, *Halidrys*, and *Sargassum* spp.	Malformations	Kohlmeyer and Demoulin, 1981
H. decidua Cribb et Cribb	*Sargassum daemedii*	Malformations	Cribb and Cribb, 1956
H. irritans (Setchell et Estee) Cribb et Cribb	*Sargassum osmundacea*, *Halidrys siliquosa*	Malformations	Cribb and Cribb, 1956
H. oceanica (Ferdinandean et Winge) Kohlm.	*Sargassum fluitans*, *S. natans*	Malformations	Kohlmeyer, 1971b
H. tumefaciens Cribb et Cribb	*Sargassum* sp.	Malformations	Cribb and Cribb, 1956
Genus Phycomelaina			
P. laminariae (Rostrup) Kohlm.	*Laminaria* spp.	Discoloration and malformation	Kohlmeyer, 1968

Table 27.1 Species of Parasitic Filamentous Fungi Present in Algae or Algal Canopies (Continued)

Marine Fungus	Alga Host	Symptom of Disease	Reference
Genus Polystigma			
P. apophlaeae Kohlm.	*Apophlaeae lyallii*	Discoloration	Kohlmeyer and Demoulin, 1981
Genus Pontogenia			
P. erikae Kohlm. et Demoulin	*Ectocarpus* sp.		Kohlmeyer and Demoulin, 1981
P. calospora (Patouillard) Kohlm.	*Castagnea chordariaeformis*		Kohlmeyer, 1975
P. codiicola (Dawson) Kohlm. et Kohlm.	*Codium mucronatum*, *C. simulans*		Kohlmeyer and Kohlmeyer, 1979
P. cubensis (Hariot et Patouillard) Kohlm.	*Halopteria scoparia*		Kohlmeyer, 1975
P. enormis (Patouillard et Hariot) Kohlm.	*Halopteria scoparia*		Kohlmeyer, 1975
P. padinae Kohlm.	*Padina durvillaei*		Kohlmeyer, 1975
P. valoniopsidis (Cribb et Cribb) Kohlm.	*Valoniopsis pachynema*		Kohlmeyer, 1975
Genus Didymella			
D. fucicola (Sutherland) Kohlm.	*Fucus spiralis*, *F. vesiculosus*, *P. canaliculata*, *E. clandestina*, *E. furcicola*		Kohlmeyer, 1968
D. gleopeltidia (Miyabe et Tokida) Kohlm.	*Gloiopeltis furcata*	Discoloration	Kohlmeyer and Kohlmeyer, 1979
D. magnei G. Feldmann–Maz.	*Palmaria palmata*		Kohlmeyer and Kohlmeyer, 1979; Kohlmeyer and Volkmann-Kohlmeyer, 2003
Genus Lautitia			
L. danica (Berless) Schatz	*Chondrus crispus*	Discoloration	Schatz, 1984

Phoma marina Lind.	Conidial state of *L. danica*		Lind, 1913; Kohlmeyer and Kohlmeyer, 1979
Genus Massarinsa			
M. cystophorae (Cribb et Herbert) Kohlm. et Kohlm.	*Cystophora retroflexa*, *C. subfarcinata*	Malformations	Kohlmeyer and Kohlmeyer, 1979
Genus Thalassoascus			
T. cystoaeirae (Oilivier) Kohlm.	"Aglaozonia," *Cystoseira*, *Zanardina* spp.	Discoloration	Kohlmeyer and Volkmann-Kohlmeyer, 1991
T. lessoniae Kohlm.	*Lessonia*		
T. tregoubovii Ollivier	*Aglazonia*, *Zanardinia*		Ollivier, 1926
Ascomycota *Incertae sedia*			
Orcadia ascophylli (Sutherland) Kohlm.	*Ascophyllum*, *Pelvetia*, *Fucus* spp.		Sutherland, 1915c; Kohlmeyer and Kohlmeyer, 1979
Basidiomycota			
Mycaureola dilseae Maire et Chemin	*Dilsea carnosa*	Discoloration	Maire and Chemin, 1922
Deuteromycota			
Gloeosporidinia cecidii (Kohlm.) B. Sutton	*Cystoseria*, *Halidrys*, *Sargassum* spp.	Hyperparasite restricted to host tissue of galls	Sutton, 1980
Found on Animal Hosts			
Laboulbenia marina Picard	On elytra or hair of *Aepus robini* living in the *Laminaria* zone		Picard, 1908; Kohlmeyer and Volkmann-Kohlmeyer, 2003
Abyssomyces hydrozoicus Kohlm.	*Chitinous hydrorhiza* and hydrocaulon of hydrozoa	Saprophytic?	Kohlmeyer, 1970; Kohlmeyer and Volkmann-Kohlmeyer, 2003

Kohlmeyer (1968, 1971, 1972, 1973b), Kohlmeyer and Kohlmeyer (1972), Jones (1976), and Andrews (1976). The last author pointed out that the increased attention focusing on algae-inhabiting fungi was led by a growing interest in marine and estuarine habitats in terms of conservation and the potential use of marine algae as a natural resource. Later studies critically investigated the mode of fungal life associated with algae. Fries (1979, 1988) reported the physiological characterization of *Mycophycias ascophylli* and the response it generated from *Ascophyllum nodosum* when growing endophytically. Schatz (1983) examined the developmental morphology and life history of *Phycomelaina laminariae*, while Garbary and Gautam (1989) and Stanley (1991) reported on the autoecology and ultrastructural interactions of algicolous fungi and their respective hosts.

The number of algicolous species described at the beginning of the last decade was 66 (Kohlmeyer and Volkmann-Kohlmeyer, 1991). This has now increased to 79 species (Kohlmeyer and Volkmann-Kohlmeyer, 2003), representing a rate of discovery of about one new species every year for the last decade, which is small compared with other fungal groups (Hawksworth, 2001). There are a number of reasons that account for this low number. During the past five decades, research in marine mycology has concentrated on lignicolous and manglicolous fungi (Kohlmeyer and Volkmann-Kohlmeyer, 2003) at the expense of other groups (Hyde et al., 2000). Algae, seaweeds in particular, are difficult to culture axenically so that *in vitro* infection studies are impractical (Kohlmeyer and Kohlmeyer, 1979). Furthermore, the majority of identified algicolous fungi are parasites or saprobes (Table 27.1, Table 27.2, and Table 27.3), often with precise growth or germination requirements, making colonization studies difficult to perform.

27.1.2 Fungal Taxonomical Groups Associated with Algae

Representatives of the Ascomycota, Basidiomycota, Labyrinthulomycota, and Oomycota can be found associated with algae; however, the distribution of species is not equal between these phyla. With only one parasite described from the Basidiomycota, *Mycaurola dilseae* infecting *Dilsea carnosa* (Porter and Farnham, 1986), and few conidial forms, the majority of filamentous fungi are ascomycetes primarily represented by Sordariomycetidae and Dothideomycetidae (Table 27.1, Table 27.2, and Table 27.3). From the Sordariomycetidae, members of the Phyllachorales and, to a much lesser extent, the Lulworthiales form the largest groups of parasites and pathogens, but their phylogenetic affiliations are unclear (Kohlmeyer et al., 2000; Kirk et al., 2001). The positions of the genera *Haloguignardia*, *Phycomelaina*, and *Polystigma* within the Phyllachorales are vague and in need of more extensive analyses, as is the exact relationship between the Spathulosporales and the genus *Pontogeneia* with other ascomycetes (Eriksson, 2002). Recent phylogenetic analysis of sequences from dried herbarium specimens has suggested that the origin of *Spathulospora* species lies within the Lulworthiales (Inderbitzin et al., 2004). Nakagiri and Ito (1997) suggested, on morphological grounds, that the species *Retrostium amphiroae* is a phylogenetic link between spathulosporalean fungi and other algae-inhabiting marine fungi of the genera *Chadefaudia* and *Haloguignardia*. Further progress, however, in defining these relationships, or developing molecular nonculturing detection assays, is hindered by the lack of sequence information.

Many algicolous fungi are found associated solely with algae and are known only from descriptions of herbarium material (Kohlmeyer and Kohlmeyer, 1979). The unitunicate species of the genera *Spathulospora*, *Chadefaudia*, *Haloguignardia*, *Retrostium*, *Hispidicarpomyces*, and *Pontogenia* are all specific to algae (Kohlmeyer and Kohlmeyer, 1979; Nakagiri and Ito, 1997), whereas members of the genera *Lindra* and *Lulworthia* are found associated with wood as well as algae. Fewer bitunicate species are associated solely with algae, such as those from the genera *Mycophycias* and *Lautitia* (Kohlmeyer and Volkmann-

Table 27.2 Submarine Lichen-Like Associations between Marine Filamentous Fungi and Macroalgae

Fungus	Algal Partner or Substrate	Type of Association	Reference
Pyrenocollema pelvetiae (Sutherl.) D. Hawksw.	Unidentified blue–green alga epiphytic on *Pelvetia*	Primitive lichen	Hawksworth, 1988
Chaudefaudia corallinarum (Crouan et Crouan) Muller et von Arx	*Dermatolithon pustulatum*, *Dermatolithon* sp., *Epilithon membranaceum* epiphytic on diverse macroalgae and sea grass leaves	Primitive lichen	Ainsworth et al., 1973
Pharcidia balani (Winter) Bauch.	Various species of microscopic algae epiphytic on calcareous shells of mollusks and cirripedes	Primitive lichen	Bauch, 1936
Pharcidia rhachians Kohlm.	Unidentified blue–green and brown algae epiphytic on *Laminaria digitata*	Primitive lichen	Kohlmeyer, 1973a
Pharcidia laminariicola Kohlm.	On stipes of *Laminaria digitata* forming a lichenoid association with epiphytic *Ectocarpus fasciculatus*	Primitive lichen	Kohlmeyer, 1973a
Leiopoloea pelvetiae (Sutherland) Kohlm. et Kohlm.	Unidentified blue–green algae on *Pelveta canaliculata*	Primitive lichen	Kohlmeyer, 1973b
Blodgettia confervoides Harvey C. fuligenosa Kohlm. et Volk-Kohlm.	*Cladophora catenta*, *Siphonocladus rigida*	Mycophycobiosis	Hawksworth, 1987
Mycophycias ascophylli (Cotton) Kohlm.	*Ascophyllum nodosum*, *Pelvetia canaliculata*	Mycophycobiosis	Kohlmeyer and Volkmann-Kohlmeyer, 1998
Septoria ascophylli Melnik et Petrov	Possible spermatial state of *Mycophycias ascophylli*		Melnik and Petrov, 1966 See Kohlmeyer and Volkmann-Kohlmeyer, 1991
Mycophycias apophlaeae Kohlm.	*Apophlaea lyallii*	Mycophycobiosis	Kohlmeyer and Volkmann-Kohlmeyer, 1998

Table 27.2 Submarine Lichen-Like Associations between Marine Filamentous Fungi and Macroalgae (Continued)

Fungus	Algal Partner or Substrate	Type of Association	Reference
Mastodia tessellata (Hooker f. et Harvey) Hooker f. et Harvey ex Hooker Kohlm. et Kohlm.	*Prasiola borealis*, *P. tessellata*	Mycophycobiosis or parasitism	Hooker, 1847 See Kohlmeyer and Volkmann-Kohlmeyer, 1991
Turgidosculum ulvae (Reed) Kohlm. et Kohlm.	*Blidingia minima* var. vexata Prasiola spp.	Mycophycobiosis or parasitism	Schatz, 1980

Kohlmeyer, 1998), and many are linked to taxonomic groups found in terrestrial environments such as those from the genera *Didymella* and *Massarina* (Kirk et al., 2001).

Other taxonomic groups containing algicolous fungi are found within the Hypocreomycetidae and include members from the Halosphaeriales and Hypocreales (Table 27.3). The Halosphaeriales comprise exclusively aquatic species that are primarily marine, but with some freshwater or estuarine species (Hyde et al., 2000). There are fewer representatives from the Hypocreales, which consist of primarily terrestrial species. Molecular information, particularly nuLSU rDNA sequences, on these organisms is more extensive than it is for algicolous fungi from other taxonomic groups (Spatafora et al., 1998; Rossman et al., 1999; Kohlmeyer et al., 2000; Kong et al., 2000; Campbell et al., 2002).

27.1.3 Range of Seaweed Supporting Fungi and Host Specificity

Filamentous fungi can colonize a variety of marine algae, but the brown and red seaweeds hold the greatest diversity. Brown algae frequently supporting fungal growth include *Fucus* species, *Pelvetia canalicultata*, *Sargassum* species, *Ascophyllum nodosum*, *Laminaria* species, and *Cystoseira* species. The following red algae are found to exhibit fungal associations: *Ballia* species, *Laurencia* species, *Palmaria palmata*, and *Chondrus crispus* (for references, see Table 27.1, Table 27.2, and Table 27.3). Green algae, in contrast, are rarely subjected to fungal infection, with only *Codium* species and *Valoniopsis pachyma* reported as being parasitized (Kohlmeyer and Kohlmeyer, 1975, 1979; Kohlmeyer and Demoulin, 1981). Kohlmeyer and Kohlmeyer (1979) postulated that the fragile nature of many marine Chlorophyceae made them unsuitable hosts for slow-growing ascomycetes, but they are involved in primitive lichen and marine lichen associations (Hawksworth, 2000).

Algal-inhabiting fungi exhibit a variety of host specificities. Some relationships, where the fungus is associated with only one algal species, are highly specific. For example, *Turgidosculum ulvae* is found only in the tissue of *Blidingia minima* var. *vexata*; *Spathulospora antarctica* is located only in *Ballia callitricha*, and *Haloguignardia cystoseirae* in *Cystoseira balearica* (Table 27.1, Table 27.2, and Table 27.3). Other fungi exhibit broader host ranges with the infected algal species belonging to a single genus, as in the colonization of *Cladophora* species by *Blodgettia bornetii*, or colonize a variety of genera inside one class of algae, as do *Chadefaudia gymnogongri*, *Didymella fucicola*, *Haloguignardia irritans*, *Lulworthia kniepii*, *Orcadia ascophylli*, *Thalassoascus tregoubovii*, and *Trailia ascophilli* (Table 27.1). Finally, there are examples where the degree of host

Table 27.3 Saprohytic Fungi Associated with Algae

Fungus	Associated Algae	Host Class	Reference
	Ascomycota		
Corollospora intermedia I. Schmidt	*Fucus vesiculosus*	Phaeophyta	Schmidt, 1969
Corollospora maritima Werdermann	*Ceramium* spp. *Fucus*, *Macrocystis*, *Sargassum* spp.	Rhodophyta Phaeophyta	Werdermann, 1922
Corollospora pulchella Kohlm., Schmidt et Nair	*Fucus vesiculosus*	Phaeophyta	Kohlmeyer et al., 1967
Corollospora angusta Nakagiri	*Fucus serratus*	Phaeophyta	Nakagira and Tokura, 1987; Zuccaro et al., 2003
Crinigera maritima I. Schmidt	*Fucus vesiculosus*	Phaeophyta	Schmidt, 1969
Lulworthia salina (Linder) Cribb et Cribb	*Fucus vesiculosus*	Phaeophyta	Cribb and Cribb, 1955
Lulworthia spp.	*Fucus vesiculosus* *Saccorhiza polyschides* *Laminaria hyperborean* *Laminaria saccharina*	Phaeophyta Phaeophyta Phaeophyta Phaeophyta	Kohlmeyer and Kohlmeyer, 1979 Kohlmeyer and Volkmann-Kohlmeyer, 1991
Pronectia laminariae (O. Eriksson) Lowen	*Laminaria* sp.	Phaeophyta	Lowen, 1990
Orbillia marina Boyd	*Ascophyllum* *Fucus* *Halidrys* spp.	Phaeophyta Phaeophyta	Smith, 1908
Pleospora gracilariae Simmons et Schatz			Kohlmeyer and Volkmann-Kohlmeyer, 1991
Pleospora pelvetiae Sutherland	*Ceramium* *Chondrus crispus* *Furcellaria lumbricalis* *Laminaria* *Pelvetia* spp.	Rhodophyta Rhodophyta Rhodophyta Phaeophyta Phaeophyta	Sutherland, 1915b
	Deuteromycota		
Asteriomyces cruciatus Moreau et Moreau ex Hennebert	*Cystoseira osmundacea* *Egregia menziesii*	Phaeophyta Phaeophyta	Hennebert, 1962
Cladosporium algarum Cooke et Massee	*Laminaria digitata*	Phaeophyta	Cooke, 1890 See Kohlmeyer and Kohlmeyer, 1979
Dendryphiella arenaria Nicot	*Sargassum* sp.	Phaeophyta	Nicot, 1958

Table 27.3 Saprohytic Fungi Associated with Algae (Continued)

Fungus	Associated Algae	Host Class	Reference
Dendryphiella salina (Sutherland) Pugh et Nicot	*Chondrus* *Furcellaria* *Laminaria* *Sargassum* spp.	Rhodophyta Rhodophyta Phaeophyta Phaeophyta	Pugh and Nicot, 1964
Phoma laminariae Cooke et Massee	*Laminaria* sp.	Phaeophyta	Cooke, 1890 See Kohlmeyer and Kohlmeyer, 1979
Phoma spp.[a]	*Fucus vesiculosus* *Macrocystis integrifolia* *Porolithon onkodes*	Phaeophyta Phaeophyta Phaeophyta	Kohlmeyer and Kohlmeyer, 1979
Stagonospora haliclysta Kohlm.	*Pelvetia canaliculata*	Phaeophyta	Kohlmeyer, 1973b
Stemyphylium gracilariae Simmons (anamorphic state of *Pleospora gracilariae*)	*Sargassum muticum*	Phaeophyta	Kohlmeyer and Volkmann-Kohlmeyer, 1991
Varicosporina ramulosa Meyers et Kohlm.	*Hypnea charoides* *Sagassum* sp.	Phaeophyta Phaeophyta	Meyers and Kohlmeyer, 1965
Sigmoidea marina Haythorn, Jones et Harrison	*Fucus serratus* *Laminaria*	Phaeophyta	Haythorn et al., 1980
Epicoccum sp.[a]	*Laminaria*	Phaeophyta	Kohlmeyer and Kohlmeyer, 1979

[a] Details in Kohlmeyer and Kohlmeyer, 1979.

specificity is relaxed; for instance, *Lindra thalassiae* can occur in the leaves of *Thalassia* species (a spermatophyte) and the air vesicles of *Sargassum* species (Kohlmeyer and Kohlmeyer, 1979).

27.1.4 Geographical Distribution

Details on the biogeography of algicolous fungi are fragmentary and are based on sporadic collections made by mycologists at local sites or remotely visited locations. It is difficult, therefore, to form an exact opinion on the accuracy of any distribution beyond a rudimentary level. Many of the algal-associated fungi occur commonly on a variety of hosts, and their distributions follow that of the seaweeds colonized. *Lautitia danica* is found throughout the geographical range of *Chondrus crispus*, and *Didymella fucicola* follows the Atlantic distribution of *Fucus vesiculosus* (Kohlmeyer and Kohlmeyer, 1979; Kohlmeyer, 1983). Occasionally the individuals of a host population support a particular fungus in only one geographical area. In a survey of algicolous fungi on *Sargassum* species from the Sargasso Sea, Kohlmeyer (1971) observed the geographical localization of *Haloguignardia oceanica* in that area, whereas *Haloguingnardia tumatifaciens* and other species from *Sargassum* species are more widely distributed. The abundance of algicolous fungi in some geographical areas appears to be low, although this might reflect a limited sampling intensity. For instance, Hyde (1985) commented on the low density of higher fungi on

algal casts from tropical and subtropical locations, and Kohlmeyer and Demoulin (1981) recorded only seven algal-inhabiting species from the Mediterranean.

Water temperature plays a role in the biogeography of seaweeds (Breeman, 1988; Lüning, 1990) and marine fungi (Jones, 2000), with some species being typically tropical, temperate, or arctic. *Spathulospora antarctica* is only found growing on *Ballia callitricha* below the 10°C isotherm, whereas the same alga is infected by four other species of *Spathulospora* above this level (Kohlmeyer, 1973c; Kohlmeyer and Kohlmeyer, 1975). Furthermore, some of the species considered as cosmopolitan may consist of physiologically and genetically adapted races. *Corollospora maritima*, an arenicolous species active in the decomposition of seaweed casts, is distributed globally but comprises temperature-adapted races (Bebout et al., 1987) that give distinct random amplified polymorphic DNA (RAPD) profiles (Roberts et al., 1996), suggesting that its geographical distribution is correlated with temperature.

27.2 ALGICOLOUS FUNGAL INTERACTIONS

27.2.1 Algal–Fungal Associations

Marine algicolous fungi have customarily been detected visually by microscopic examination of algal tissue, which is either damaged, dead, or in a state of decomposition. The identification of fungi as algicolous, therefore, has relied upon using selected thalli that display pathologies. This has led to a general conclusion that fungi associated with algae, rather than other substrates, are rare and primarily parasitic, pathogenic, or saprophytic (Vrijmoed, 2000). In terms of the number of algicolous fungi recorded, this statement is true. Table 27.1, Table 27.2, and Table 27.3 list fungi associated with algae according to lifestyle and show a predominance of parasitic forms that, together with the mutualistic or commensalistic associations, form important evolutionary and ecological aspects in the adaptation of organisms to harsh environments (Stachowicz, 2001).

The degree of parasitism varies greatly in fungal infections of seaweeds. Kohlmeyer (1974) classified the effects of parasitism into three broad categories (Table 27.1): (1) weak parasitism where the infection does not alter the outer appearance of the hosts, (2) fungal infections that induce a color change in the host (the discoloration may be mild to severe and is caused by tissue disruption), and (3) the induction of malformations, such as galls. There are examples of tissue specificity involved in parasitism of algae by filamentous fungi. Lesions may be localized, such as the infection of *Sargassum* species by *Lindra crassa*, where hyphae and ascomata develop only in algal vesicles (Tubaki, 1969; Kohlmeyer and Kohlmeyer, 1979), or merged throughout the thallus. Intercellular hyphae of *Lulworthia kneipii*, which causes white spots, spread within the middle lamella of *Lithophyllum* and *Pseudolithophyllum* species and destroy neighboring algal cells without penetrating the calcareous wall (Kohlmeyer, 1969). The ability of this parasite to obtain nutrients without hyphal invasion remains unexplained. Kohlmeyer and Kohlmeyer (1979) postulated a nutrient transport system to explain the damage of chloroplasts in adjoining hyphal-free cells of *Ballia* species infected by *Spathulospora* species, which typically penetrate only one algal cell. Little evidence exists, however, to explain the mechanisms of such a system. Other examples of tissue specificity may have a chemical basis. Infections of *Chondrus crispus* by *Lautitia danica* are confined to the carposporangial and tetrasporangial pustules, which differ in the composition of carrageenans compared with vegetative tissues (Chopin et al., 1987).

The role of marine filamentous fungi in the decomposition of algal material is considered to be of minor importance (Chesters and Bull, 1963; Kohlmeyer and Kohlm-

eyer, 1979; Haythorn et al., 1980). Actinomycetes and other bacteria, yeasts, and thraustochytrids, which grow faster than filamentous ascomycetes, are reported as the primary decomposers of dead algae (Seshadri and Sieburth, 1975; Bremer, 1976). Nevertheless, a small number of marine fungi, which are capable of utilizing laminarin, cellulose, and other algal products, are routinely found on brown algal and, less frequently, on red algal casts. Arenicolous species, such as those from the genera *Corollospora*, *Asteromyces*, and *Varicosporina*, are usually considered general saprophytes using dead organic material wherever their spores may land. Species belonging to these genera can be found attached to siliceous and calcareous substances (Höhnk, 1954; Kohlmeyer, 1969) from where their hyphae may penetrate and use other substrates such as washed-up algae, leaves, rhizomes of sea grasses, or driftwood (Kohlmeyer and Kohlmeyer, 1979). A number of arenicolous fungi, such as *Corollospora angusta* (Nakagiri and Tokura, 1987), are described as such solely on the basis of their ascocarp structures and are only known from collections of sea foam (Kohlmeyer and Kohlmeyer, 1979). Thus, the distribution of these species on potential shore substrates is unknown. Other fungi that may be cultured from seaweed casts include terrestrial and facultative marine species, particularly if the algae are washed up on the upper shore.

Mutualism is an important enabling mechanism in evolutionary biology, allowing lateral gene transfer and the creation of new species via symbiogenesis (Margulis, 1992). Many examples of mutualistic associations exist in the aquatic environment where algal–fungal interactions eventually lead to permanent associations such as lichen formation, where the photobiont may be a cyanobacteria or a green alga. Although poorly understood, in the marine environment other lichenoid associations exist (Table 27.2) and include the formation of primitive lichen states (Kohlmeyer, 1973a, 1973b, 1973c; Kohlmeyer and Kohlmeyer, 1979) and mycophycobiosis (Gimmler, 2001; Kohlmeyer and Kohlmeyer, 1972, 1979). Primitive lichen states exist when the fungus lives in a loose lichenoid association occurring on epiphytic algae that grow on the surface of macroalgae, rocks, or mollusks (Kohlmeyer and Kohlmeyer, 1979). *Mycophycobiosis* is defined as "a systemic association between a fungus and a macro-algae, in which the habit of the macroalgae dominates" (Kohlmeyer and Kohlmeyer, 1979). These types of associations are characterized by facultative commensalistic and mutualistic relationships that may reflect the first phase of terrestrial associations (Hawksworth, 1988; Jorgensen, 1993).

The extent of fungal endosymbiotic relationships with seaweeds is limited to the examples of mycophycobiosis (Table 27.2). In *Mycophycias ascophylli* infections a dependency exists on the autotrophic partner, *Ascophyllum nodosum*, for nutrients (Kingham and Evans, 1986) and the fungus remains associated throughout its life cycle, exhibiting a synchronized reproduction during which the sporocarps are localized to the receptacles (Stanley, 1991). The fungus can be maintained in culture without forming fruiting structures. Its anamorph form, *Septoria ascophylli*, can be induced to sporulate; however, the spores do not germinate on artificial media (Stanley, 1991). Garbary and Gautam (1989) recorded maximum hyphal density for *M. ascophylli* in the apex, followed by the midthallus and receptacles, with the lowest in the old thallus of *A. nodosum*. Large hyphal nodes form in the intercellular spaces of the host cortex, and these may function as nutrient and metabolite distribution/collection sites. Hyphae can also penetrate into the cell walls of rhizoids from *Polysiphonia lanosa*, an obligate epiphytic red alga, that is embedded in *A. nodosum*. When this occurs, the two algae are linked together by the fungal hyphae (Garbary and MacDonald, 1995). Benefits for the algae in such symbiotic relationships can be difficult to detect, though Garbary and MacDonald (1995) provided experimental evidence that infected thalli were longer and had a greater apical diameter and more apical hairs than uninfected thalli, and that *M. ascophylii* may protect *A. nodosum* from desic-

cation (Garbary and London, 1995). Other examples of mycophycobiosis, which have been less extensively studied, vary in the degree of life cycle synchronization. The difficulty of culturing the mycobiont suggests a dependency on the photobiont that is comparable with the behavior exhibited by the fungal partners of lichens, which are often readily cultured, but with reduced growth in pure culture (Fries, 1988).

27.2.2 Fungal Colonization of Seaweeds

The canopy of seaweeds offers a protected area in an environment that is poor in nutrients and exposed to stress factors such as repeated desiccation, extreme temperature variations, sun radiation, and changes in salinity. Healthy algae release a proportion of the carbon they fix via photosynthesis as extracellular products in solution or as mucilage (Fogg, 1962; Jones and Cannon, 1986; Jones, 1988), which possibly acts as a microbial attractant. The area around the algae, which is rich in carbohydrates, lipids, peptides, and vitamins, as well as growth inhibitors such as antibiotics and phenolic compounds, resembles the rhizosphere of higher plants and therefore has been called phycosphere (Bell and Mitchell, 1972). It is within this environment that fungal–algal interactions commence with spore attachment and hyphal invasion, leading to colonization and the establishment of either a parasitic, mutualistic, or saprophytic relationship.

Ascospores, basidiospores, and conidiospores released from algal thalli are washed into the sea. The presence of spore appendages, characteristic of many marine fungi, assists in the entrapment of the propagule inside air bubbles that gather in sea foam (Kohlmeyer and Kohlmeyer, 1979; Jones, 1994), but germination does not occur at this stage. Germ tubes only form, often within a few hours, when the spores are deposited on a suitable host. Many marine fungi, such as *Lulworthia medusa* and *Corollospora maritima*, produce mucilaginous sheaths when attached to surfaces (Rees and Jones, 1984; Hyde et al., 1986), thereby promoting initial colonization. Little is known about hyphal penetration during the colonization of algae by fungi. Inter- and intracellular hyphae are observed in seaweed thalli infected by a variety of algicolous fungi. Stanley (1991) noted the formation of specialized penetration hyphae during the infection of *Dilsea carnosa* by *Mycaureola dilseae*. These occurred as fine hyphal extensions occasionally forming bifurcate tips that entered the host cells in two proposed stages: (1) the enzymatic degradation of the cell wall and (2) the exertion of mechanical pressure at the point of contact. The same mechanisms are used to infect higher plants (Agrios, 1997). Other points of entry for hyphal infection include sites of damage caused by the action of epiphytic animals such as tube-forming annelids and polychaetes (Kohlmeyer and Kohlmeyer, 1979).

The colonization of a living organism by another requires a series of interactions that are more complicated than simple settlement onto inorganic or dead material. Recognition is clearly a prerequisite in the initiation of a parasitic or symbiotic relationship. Some algicolous fungi, such as *Sigmoidea marina*, actively grow toward algal substrates showing a degree of recognition (Zuccaro et al., 2001). Such responses are essential in forming signal exchange mechanisms that may begin with a solute "dialogue" in the water (Reisser, 1992). Colonization often involves the evolution of specialization, which may incur a cost to the invading species (Steinberg and de Nys, 2002).

The degree of microbial colonization of seaweeds varies according to the species; some suffer acute biofouling, while others remain free from attack. *Pelvetia limitata* and *Pelvetia canaliculata*, which are upper-shore seaweeds that frequently undergo desiccation, become heavily colonized by epiphytes after prolonged submergence at the lower shore (Norton, 1994). Other seaweeds thrive on regular periods of submergence, which reduces exposure to the sun and desiccation, and show little sign of colonization. The differences between the responses to microbial colonization reflect the evolution of different protection

mechanisms by algae, which include mechanical processes such as sloughing (McArthur and Moss, 1977; Moss, 1982) and chemical defenses (Van Alstyne et al., 2001).

The surface of macroalgae, although providing nutrients, space, and protection, can also offer a barrier to colonization when growth-inhibiting metabolites are secreted into the phycosphere. Such chemical defense metabolites may be provided by the algae or by epiphytic organisms (Armstrong et al., 2001). The screening of aqueous, ethanolic, or dichloromethane tissue extracts from different algal species for bioactive compounds identified the presence of antimicrobial compounds, many of which are novel (Hornsey and Hide, 1974, 1976; Tariq, 1991; Hellio et al., 2000; Kubanek et al., 2003). In general, brown and red algae exhibit a high bioactivity against fungi as well as against bacteria and diatoms (Fletcher, 1975), whereas green algal extracts appear to be mostly active against gram-positive and marine bacteria (Table 27.4). These antimicrobial profiles may be specifically antagonistic to the group of colonizing organisms that attack the different species of algae. Only a few green algae support the slow growth of filamentous fungi, probably because their short life cycles do not offer adequate development time for colonization to be established, whereas they often suffer degradation by faster-growing organisms such as bacteria and yeast, particularly toward the end of their growth season. Hornsey and Hide (1974) detected antibacterial activity in *Ulva lactuca* extracts only during autumn and winter, and observed three categories of seasonal variation in bioactivity from a range of seaweeds: (1) uniform activity throughout the year (e.g., *Polysiphonia lanosa*), (2) a conspicuous period of inactivity such as that shown by *Laminaria saccharina*, and (3) a conspicuous peak of activity at one period of the year (e.g., *Laminaria digitata*).

Perenial, multicellular macroalgae provide a substrate for fungal colonization, with high structural complexity that offers good protection from environmental stress and great longevity, allowing the establishment of fungal colonies. The ability to embed into a tougher structure affords many organisms a means of avoiding removal by sloughing mechanisms (Gonzalez and Goff, 1989). Although there is no direct evidence that a successful fungal infection relies upon embedment, many fungi excrete extracellular enzymes, such as laminarinases and cellulases, that may damage tissue permitting hyphal invasion (Haythorn et al., 1980; Stanley, 1991). The presence of identified fungicidal metabolites produced by algae provides indirect evidence that fungal colonization of healthy seaweed thalli may occur regularly. Kubanek et al. (2003) isolated and characterized a 22-membered cyclic lactone, lobophorolide from *Lobophora variegata* with sub-micromolar activity against the marine, algicolous fungi *Dendryphiella salina*, and *Lindra thalassiae* (Table 27.2 and Table 27.3).

Depending on the algal species examined, bioactivity can vary between tissue types due to different allocation strategies of metabolite resources. Hornsey and Hide (1976) recorded variation in bioactivity between tissues for different seaweeds. *Chondrus crispus*, *Dilsea carnosa*, and *Codium fragile* exhibited maximum activity in meristematic tissues, whereas *Ulva lactuca* showed a uniform distribution throughout the thallus and *Laminaria saccharina* had the greatest activity in the oldest parts of the thallus. Preliminary results from experiments designed to assay different body parts for bioactivity suggest that this might also be true for *Fucus serratus* (Zuccaro, unpublished data). Hornsey and Hide (1974) examined extracts from a variety of algae, describing the Fucaceae as a group with low bioactivity. In general, dichloromethane extracts from tissues of all body parts of *F. serratus* exhibit little activity except against *Chlorella fusca* (Chlorophyceae) and a pronounced activity against *Mycotypha microspora* (Zygomycota) in the growing tips (Table 27.5).

Microbial colonization can also be induced by metabolites produced by seaweeds, but no information exists on filamentous fungi that recognize these different elicitors or receptors. Many algicolous fungi are host-specific parasites, and it might be expected that

Table 27.4 List of Antimicrobial Activity of Algal Extracts

	Algal Group	Antifungal Activity	Antibacterial Activity		Extraction Methods	References
			Gram +	Gram –		
Ulva lactuca[a]	Chlorophyta	–	–	–	Aqueous; ethanol; dichlorometane	Hellio et al., 2000
Enteromorpha intestinalis	Chlorophyta	–	+	–	Ethanol	Hellio et al., 2000
Cladophora rupestris	Chlorophyta	–	–	–	Aqueous; ethanol; dichlorometane	Hellio et al., 2000
Lobophora variegata	Phaeophyta	+	nt	–	Methanol-dichlorometane 1:1	Kubanek et al., 2003
Pelvetia canaliculata	Phaeophyta	–	–	–	Aqueous; ethanol; dichlorometane	Hellio et al., 2000
Fucus vesiculosus	Phaeophyta	–	–	–	Aqueous; ethanol; dichlorometane	Hellio et al., 2000
Ascophyllum nodosum	Phaeophyta	+	–	–	Ethanol; dichlorometane	Hellio et al., 2000
Sargassum muticum	Phaeophyta	+	–	+	Ethanol	Hellio et al., 2000
Laminaria ochroleuca	Phaeophyta	+	–	–	Ethanol	Hellio et al., 2000
Ectocarpus siliquosus	Phaeophyta	–	–	–	Aqueous; ethanol; dichlorometane	Hellio et al., 2000
Dilsea carnosa	Rodophyta	+	nt	nt	Ethanol	Tariq, 1991
Laurencia pinnatifida	Rodophyta	+	nt	nt	Ethanol	Tariq, 1991
Odonthalia dentata	Rodophyta	+	nt	nt	Ethanol	Tariq, 1991
Polysiphonia lanosa	Rodophyta	+	nt	nt	Ethanol	Tariq, 1991
Chondrus crispus	Rodophyta	–	nt	nt	Ethanol	Tariq, 1991
Chondrus crispus[b]	Rodophyta	+	–	–	Ethanol	Hellio et al., 2000
Laurencia pinnatifida	Rodophyta	+	+	–	Ethanol; dichlorometane	Hellio et al., 2000
Polysiphonia lanosa	Rodophyta	–	+	+	Ethanol; dichlorometane	Hellio et al., 2000
Ceramium rubrum	Rodophyta	–	–	–	Aqueous; ethanol; dichlorometane	Hellio et al., 2000
Cryptopleura ramosa	Rodophyta	+	–	–	Dichlorometane	Hellio et al., 2000
Laurencia rigida	Rodophyta	+	–	–	Dichlorometane	König and Wright, 1997

Note: nt: not tested.

[a] Hornsey and Hide (1976) recorded an activity against staphylococcus aureus in autum and winter.

[b] Hornsey and Hide (1974) detected a considerable antibacterial activity of Chondrus crispus and the lack of it in Mastocarpus stellatus.

Table 27.5 Fucus Serratus Bioactivity

	Radius of Zone of Inhibition (mm)							
	Bacteria		Fungi					Alga
Fucus Parts	Ba. Meg.	E. coli	M. viol.	M.m.	D.s.	Fus.	A.c.	Chlor.
Receptacles	—	—	~ 0.3	~ 0.1	—	—	—	0,5
Tips	~ 0.2	—	~ 0.5	0,2	—	—	—	0,5
Foot	—	—	—	—	—	—	—	0,5
Body	~ 0.2	—	~ 0.8	~ 0.1	—	—	—	0,5

Note: Different algal tissues were algal material extracted with ethyl acetate (1 mL/g) overnight. The crude extracts of different parts of *Fucus serratus* were then dissolved in methanol-acetate (1:1) and tested as described by Schulz et al., 1995. Ba. meg.: *Bacillus megaterium,* E. coli: *Escherichia coli,* M. viol.: *Microbotryum violaceum,* M.m.: *Mycotypha microspora,* D.s.: *Dendryphiella salina,* Fus.: *Fusarium oxysporum,* A.c.: *Asteromyces cruciatus,* Chlor.: *Chlorella fusca,* partial inhibition zone.

the chemical cues for colonization would also be host specific. In the pathogenic invasion of red algae by some oomycetes the chemical cues for infection are specific carbohydrates. *Pythium porphyrae* infects *Porphyria yezoensis*, causing red rot disease, via a complex series of interactions after zoospore attachment, including encystment on the thallus surface and cyst germination followed by penetration. The formation of appressoria and subsequent infection pegs is crucial for penetration, and these stages are controlled by signals from the algal thalli (Kerwin et al., 1992). Zoospores of *P. porphoryrae* attach specifically to red algal species, such as those of *Porphyra* and *Bangia*, containing characteristic sulfated galactans (porphyran). Although porphyrans are present in the cuticles of other red algae, encystment and formation of appressoria only occur in the presence of a sulfated galactan, a substituted agar, found in species of *Porphyra* and *Bangia* (Morrice et al., 1983). This molecule contains 3,6-anhydrogalactose and 6-O-methyl-D-galactose residues similar to those found in agars and galactose-6-sulfate residues that resemble carrageenans. The attachment and encystment of zoospores can be induced *in vitro* after settlement on agarose films or on agar; however, appressoria formation only occurs in the presence of extracted sulfated porphyran from *Porphyra* species (Uppalapati and Fujita, 2000). In this case, the colonization process follows an initial broad approach where zoospores can attach to a large variety of red algae, but not green or brown, then specializes when the appressoria are formed after recognition of a characteristic algal substance.

Finally, chemical inhibitors present in the phycosphere may mediate the stimulation of fungal growth if their concentration is very low. The stimulation of biological processes by low doses of toxicants is defined as hormesis (Luckey et al., 1975). Dilute ethanolic extracts from *Hypnea spicifera* promote colony growth of *Rhizoctonia solani* (Barreto et al., 1997), but inhibit it when doses are higher. Extracts from red and brown algae (*Osmundaria serrata* and *Zonaria subarticulata*) produced either hormetic or inhibitory effects on the growth of two fungal pathogens, *Colletotrichum gloeosporoides* and *Rhizoctonia solani*, depending on the concentration (Barreto et al., 2002). The mechanisms for hormesis are unknown, and it is conceivable that there are solvent or micronutrient effects involved in the phenomenon (Stebbings, 1982, 1997).

27.3 FUNGAL DIVERSITY ASSOCIATED WITH *FUCUS SERRATUS*

Populations of *Fucus serratus* occupy the lower shore of sheltered or semiexposed coasts in temperate regions and have a well-described ecology that is suitable for investigating fungal interactions in a natural environment. The treatment and examination of algal samples do not differ significantly from those applied to other substrates in mycological studies; however, the identification of a fungal–seaweed association often requires direct observation and sectioning of many specimens (Kohlmeyer and Kohlmeyer, 1979). Unfortunately, the time-consuming examination of many algal thalli can fail to identify fungi that do not, or rarely, form obvious sporulation structures or that exist with multiple life cycle morphologies. Indirect culturing techniques are considered less reliable than the above as methods for detecting associations, or estimating diversity, because a wide range of environmentally unrelated or nonassociated fungi may be preferentially cultured (Hyde et al., 2000). Furthermore, antagonistic interactions between isolates during plating and overgrowth of algal segments by fast-growing fungi may bias the isolation of algicolous fungi. Molecular ecology techniques provide a means to identify the principal fungal populations that are active in an environment (Kowalchuk, 1999), complementing the use of classical methods. The following section contains the first model study in which the fungal community associated with *F. serratus* is described for healthy and decomposing fronds using molecular and conventional culturing techniques.

27.3.1 Sampling Strategies

Thirty-nine specimens of submerged, attached *Fucus serratus* and nine casts were collected from a rocky-shore site on Helgoland Island, Germany, over the course of 1 year on five separate occasions. All the thalli collected were cleaned with sterile water containing the detergent sodium lauryl sulfate. One portion of the healthy thalli was surface sterilized to identify potential endophytes. A total of ca. 3500 disks were excised aseptically from thalli for the culture isolation study. Six different media, all containing artificial seawater and antibiotics, were used for the culturing analysis, including oliogotrophic and *Fucus* extract media (Zuccaro et al., 2003). DNA extracted from a total of 320 g of algal material was diluted for PCR-DGGE (polymerase chain reaction denaturing gradient gel electrophoresis) analysis of nuSSU and nuLSU fragments to generate fungal community profiles (Figure 27.1). Each analysis involved multiple PCRs so that the variation in the fungal species amplified for each sample could be monitored. In this way, the prevalent fragments detected would represent fungi with the highest biomass and, therefore, be active members, rather than nonactive propagules that would be expected to occur at much lower concentrations (Zuccaro et al., 2003).

Two sampling strategies incorporating both molecular and fungal culturing techniques were employed to identify the main populations associated with *F. serratus*:

Whole thalli analysis: Disks cut randomly from intact thalli were either plated on to media for fungal isolation or extracted for DNA analysis. After PCR-DGGE analysis and molecular cloning of PCR products, fungal sequences from DGGE bands and cloned fragments were compared with those from the isolates to obtain identity matches for the prevalent ascomycete populations. The profiles of individual thalli were compared to gauge the community complexity and frequency of isolate occurrence.

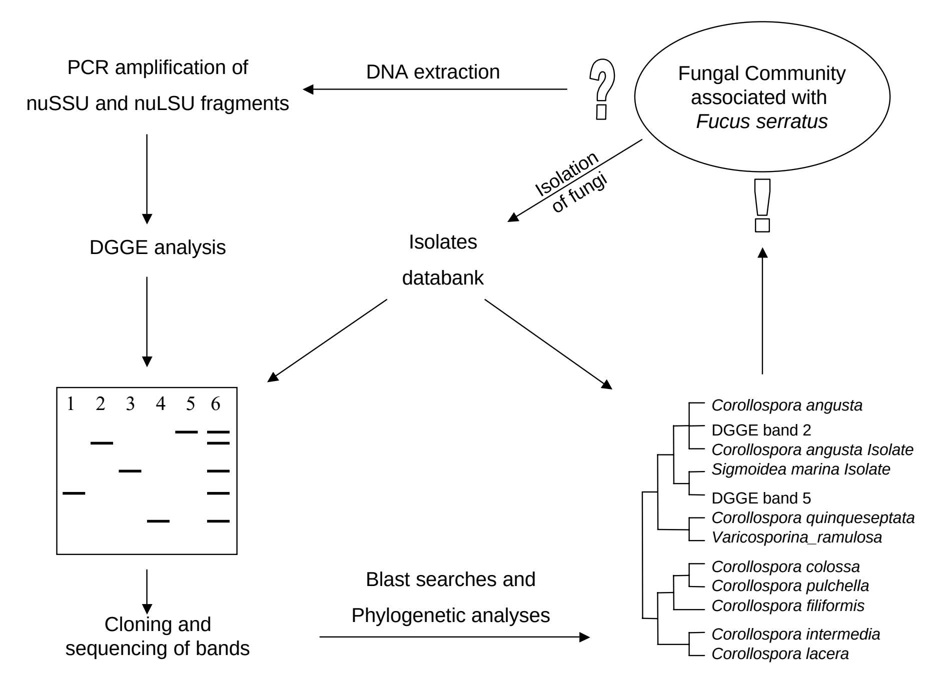


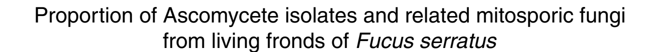
Figure 27.1 Analysis of nuSSU and nuLSU fragments to generate fungal community profiles.

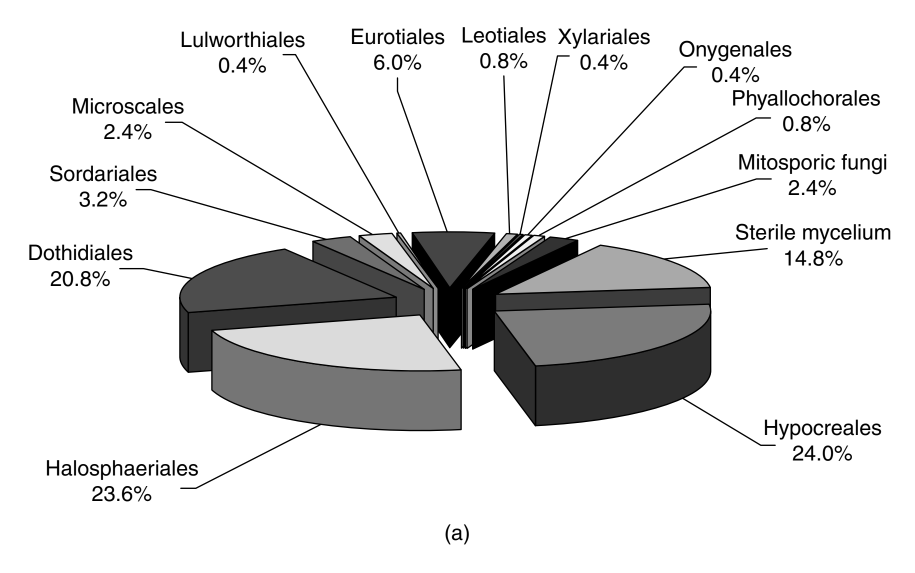
Sectioned thalli analysis: Thalli were sectioned into four parts representing the growing tips, receptacles, foot, and main body. The body sections were pooled from all of the thalli sampled and used to cultivate isolates or extract DNA. The fungal community profiles generated after molecular analysis reflected the populations active in localized regions of the thalli (fungal vertical distribution, Section 27.4.2).

27.3.2 Fungal Isolate Diversity

Few records detail the fungal colonization of healthy fronds, with most reports describing parasites and saprobes of casts or diseased fronds. Over a 3-year period, Haythorn et al. (1980) recorded six fungal species from *Fucus serratus* casts: *Dendryphiella salina*, *Alternaria marina*, *Asteromyces cruciatus*, *Sigmoidea marina*, and unidentified species of *Penicillium* and *Doratomyces*. This low diversity was reflected in collections they made from other seaweed casts with only 19 species (52 isolates) observed growing from 10 different species of seaweeds. The diversity of fungi associated with casts of *F. serratus* during our study, with 84 isolates, represented six orders of the Ascomycota (Figure 27.2b). Species from the Dothideales formed the prevailing group, with representatives such as *Dendryphiella salina* and species of *Phoma*, *Cladosporium*, and *Alternaria*. Other orders observed included the Hypocreales, with isolates from the genera *Acremonium*, *Fusarium*, and *Trichoderma*; the Halosphaeriales; and the Lulworthiales. Isolates from the last two orders were identified as *Corollospora angusta*, *Corollospora intermedia*, and *Lindra obtusa*.

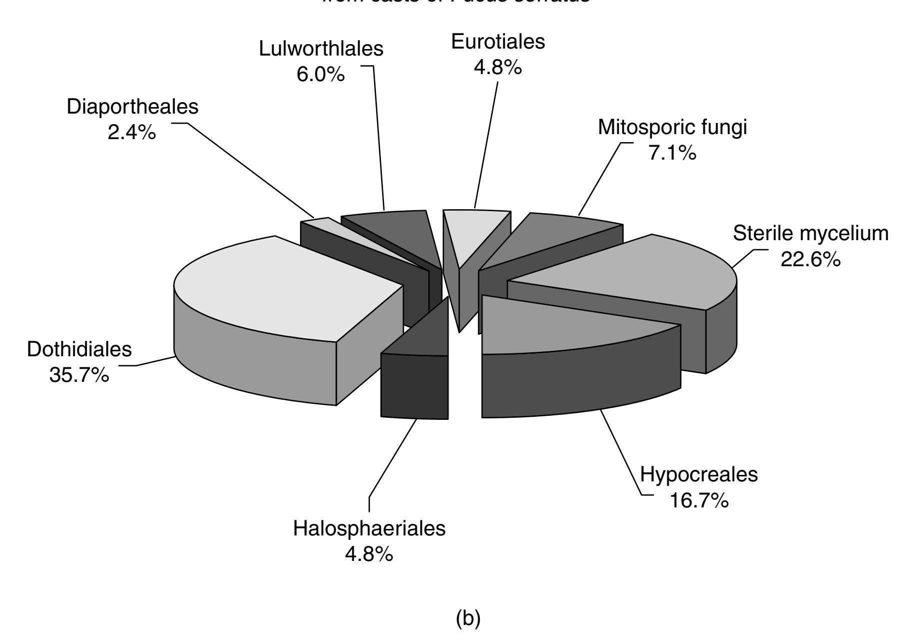
The number of isolates recovered from healthy fronds totaled 250 representing 10 orders, with members of the Hypocreales, Halosphaeriales, and Dothideales predominating (Figure 27.2a). Isolates recovered with a high frequency included those of *Sigmoidea marina* and *Acremonium fuci*, with summer (July) and autumn (October) retrieval peaks, respectively. *Dendryphiella salina* and species of *Fusarium*, *Cladosporium*, and





(a)

Proportion of Ascomycete isolates and related mitosporic fungi
from casts of *Fucus serratus*



(b)

Figure 27.2 Proportion of Ascomycete isolates and related mitoscopic fungi from living fronds of (a), casts of (b), and dead (c) *F. serratus*. (Continued)

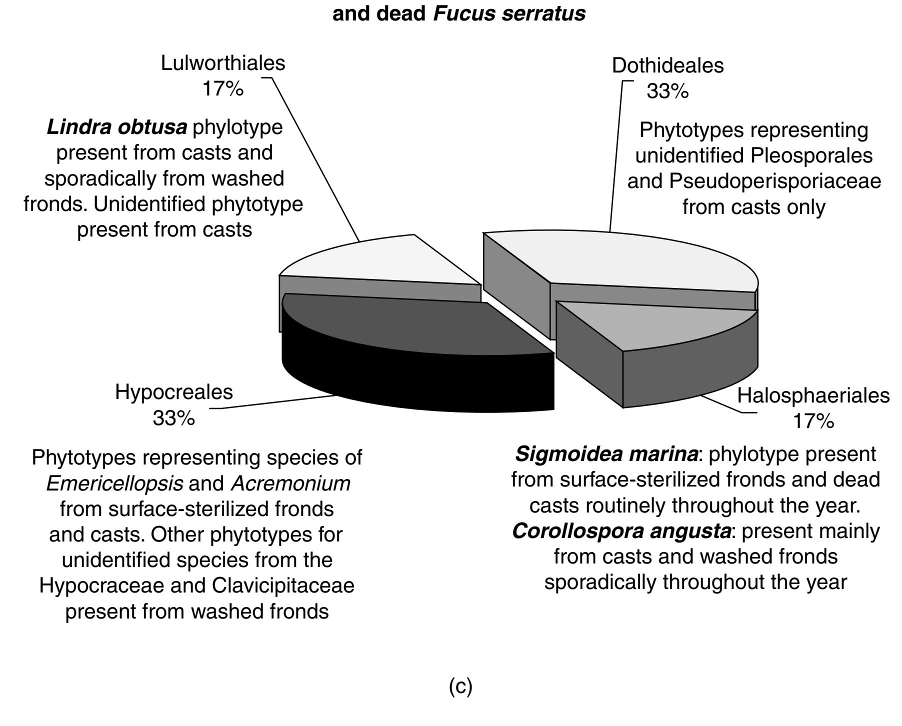


Figure 27.2 Continued.

Penicillium were also routinely recovered, as were isolates from *Trichoderma*, *Phoma*, and *Paecilomyces*. The greater diversity of ascomycetes cultured from healthy fronds (Figure 27.2a and b) is perhaps surprising considering the presence of protection mechanisms (Section 27.2.2) that might limit fungal growth. However, the increased diversity probably reflects a bias in the sampling effort as fewer casts were collected and examined. Seasonal differences in the rate of isolate retrieval were observed, with the lowest recorded in January, when the seawater temperature was coldest. The highest species diversity was observed in October, which is analogous to higher plant colonization (Petrini, 1991; Stone et al., 2000). When the algal tissue was surface sterilized, fewer isolates were recovered and the proportion of prevailing orders changed with the Halosphaeriales forming the largest group. This was due entirely to the recovery of *Sigmoidea marina* isolates, which were routinely retrieved throughout the year. *Acremonium fuci*, a conidial isolate linked to the Hypocreales (Zuccaro et al., 2005), was also frequently isolated. A large proportion of the isolates were recovered as sterile mycelium (Figure 27.2a and b) from algal material, some of which were characterized molecularly.

27.3.3 Molecular Approaches to Assessing Fungal Diversity

Molecular techniques allow assessments of diversity without culturing, providing information on community structure (rDNA/rRNA profiles) and function (gene transcripts) directly from the environment (Torsvik and Øvreås, 2002). The retrieval and identification of fungal sequences from environmental samples require the application of PCR or hybridization systems to isolate taxonomic groups (Amann et al., 1995) and methods to separate the different nucleic acid fragments from each other. There is no molecular information on the diversity of fungi associated with seaweeds or other algae. In this section, the

molecular techniques used to detect fungi in the environment are reviewed and a strategy, along with its implementation, for detecting algicolous fungi described, which resulted in the molecular identification of fungi associated with the canopy of *F. serratus*.

27.3.3.1 PCR Primers and Genetic Systems

The main targets for primer design are the ribosomal RNA genes and spacer regions. The conserved and variable domains of these genes provide phylogenetic information for taxonomic studies and sequence identification (Horton and Bruns, 2001). Various polymerase chain reaction (PCR) primers have been used to amplify fungal sequences from environmental samples (Table 27.6), primarily targeting the nuSSU ribosomal RNA gene. The nuSSU rDNA primers are capable of amplifying sequences from a broad range of fungal phyla; however, not all of them are fungal specific (Borneman and Hartin, 2000; Zuccaro et al., 2003), and some exhibit a bias during amplification (Anderson et al., 2003b). Few primers are phyla specific. The greater sequence variations observed within the ITS-5.8S rDNA and, to a lesser extent, the 28S rDNA regions permit the design of taxon-specific primers, allowing environmental analyses to be focused on specific groups of fungi (Cullings and Vogler, 1998). Primers targeting basidiomycetes and ascomycetes have been described for the ITS-5.8S rDNA region (Gardes and Bruns, 1993; Larena et al., 1999) and mycobiont-specific primers for the nuLSU rRNA gene (Zoller et al., 1999; Döring et al., 2000). Of these two genetic regions, the 28S rDNA is more generic, allowing the identification of sequences to the genus level (van Tuinen et al., 1998; Taylor and Bruns, 1999), and is potentially useful for broad-based identifications. ITS sequences can be extremely variable, allowing discrimination between species and even strains, but can contain repeat motifs or deleted segments, making primer design difficult within these regions. Taylor and Bruns (1999) circumnavigated this problem by designing primers that amplified the ITS-5.8S-28S region from conserved domains of the 18S and 28S rRNA genes. Few other genetic systems have been used to assess fungal molecular diversity. Lyons et al. (2003) identified a novel fungal lineage by comparing laccase gene sequences; however, the use of other genetic regions in environmental studies is limited by the availability of sequence information in databases.

27.3.3.2 Molecular Separation and Identification

Mixed-template PCR products can be separated by molecular cloning (Horton and Bruns, 2001) or by using a variety of techniques that fractionate according to size (amplified rDNA restriction analysis, ARDRA; ribosomal intergenic spacer analysis, RISA; automatic ribosomal intergenic spacer analysis, ARISA; terminal restriction fragment length polymorphism, T-RFLP) or sequence (denaturing gradient gel electrophoresis, DGGE; temperature gradient gel electrophoresis, TGGE) (Kowalchuk, 1999; Osborn et al., 2000; Øvreås, 2000; Delong, 2001; Ranjard et al, 2001; Gomes et al., 2003). In reality, molecular cloning should accompany all methods, as this allows estimates of PCR bias to be made. Comparisons between cultured isolates and environmental signals can be performed with all the methods, but the profiling techniques, such as T-RFLP, allow a large number of samples to be processed quickly for complex environments. Environments with a low density of species diversity can be studied using DGGE and TGGE techniques (Muyzer, 1999). The main microbial populations are visualized as bands after electrophoresis, which typically account for 0.1 to 1% of the total organisms that may be targeted (Muyzer et al., 1993; Murray et al., 1996). Banding profiles between samples can be compared (Muyzer and Smalla, 1998; Fromin et al., 2002), but each band should be characterized by cloning and sequencing

Table 27.6 Sequence of Primer Pairs Used in Environmental Studies and Their Fungal Specificity

Primer	Sequence	Fungal Specificity	Reference
	nuSSU RNA Gene		
NS1[a]	CCAGTAGTCATATGCTTGTC	No[h]	White et al., 1990
NS8[a]	TCCGCAGGTTCACCTACGGA		White et al., 1990
NS1[a]	CCAGTAGTCATATGCTTGTC	NT	White et al., 1990
NS2+10	GAATTACCGCGGCTGCTGGC		Kowalchuk et al., 1997
EF4f	GGAAGGGRTGTATTTATTAG	No[g]	Smit et al., 1999
EF3r	TCCTCTAAATGACCAAGTTTG		Smit et al., 1999
NS2f[a]	GGCTGCTGGCACCAGACTTGC	No	White et al., 1990
fung5	GTAAAAGTCCTGGTTCCCC		Smit et al., 1999
EF4f	GGAAGGGRTGTATTTATTAG	No	Smit et al., 1999
fung5	GTAAAAGTCCTGGTTCCCC		Smit et al., 1999
EF4f	GGAAGGGRTGTATTTATTAG	No	Smit et al., 1999
NS3-GC[a]	GGCTGCTGGCACCAGACTTGC		White et al., 1990
NS1[a]	CCAGTAGTCATATGCTTGTC	No[b,h]	White et al., 1990
FR1	AICCATTCAATCGGTAIT		Vainio and Hantula, 2000
FR1GC	AICCATTCAATCGGTAIT	No[f]	Vainio and Hantula, 2000
EF390	CGATAACGAACGAGACCT		Vainio and Hantula, 2000
YUNIV1	GGGAGCCTGAGAAACGGCTACCAC	NT	Hernán-Gómez et al., 2000
YUNIV3	TTCAACTACGAGCTTTTTAA		Hernán-Gómez et al., 2000
nu-SSU-0817	TTAGCATGGAATAATRRAATAGGA	No[e]	Borneman and Hartin, 2000
nu-SSU-1196	TCTGGACCTGGTGAGTTTCC		Borneman and Hartin, 2000
nu-SSU-0817	TTAGCATGGAATAATRRAATAGGA	NT	Borneman and Hartin, 2000
nu-SSU-1536	ATTGCAATGCYCTATCCCCA		Borneman and Hartin, 2000
NS26	CTGCCCTATCAACTTTCGA	NT	Gargas and DePriest, 1996

518GC	ATTACCGCGGCTGCTGG		Neefs et al., 1990
EF4	GGAAGGGRTGTATTTATTAG	NT	Smit et al., 1999
518GC	ATTACCGCGGCTGCTGG		Neefs et al., 1990
NS1[a]	GTAGTCATATGCTTGTCTC	NT	White et al., 1990
GCfung	ATTCCCCGTTACCCGTTG		May et al., 2001
F1300	GATAACGAACGAGACCTTAAC	NT	Nikocheva et al., 2003
Primer D[b]	CYGCAGGTTCACCTAC		Elwood et al., 1985
NS5[b]	AACTTAAAGGAATTGACGGAAG	NT	White et al., 1990
Primer D[b]	CYGCAGGTTCACCTAC		Elwood et al., 1985
AU2	TTTCGATGGTAGGATAGDGG	NT	Vandenkoornhuyse et al., 2002
AU4	RTCTCACTAAGCCATTC		Vandenkoornhuyse et al., 2002
	nuLSU RNA Gene		
NL209	AAGCGCAGGAAAAGAAACCAACAG	Ascomycete[c]	Zuccaro et al., 2003
NL912	TCAAATCCATCCGAGAACATCAG		Zuccaro et al., 2003
NL359	GGACGCCATAGAGGGTGAGAGC	Ascomycete[c]	Zuccaro et al., 2003
NL912-GC	TCAAATCCATCCGAGAACATCAG		Zuccaro et al., 2003
P1	ATCAATAAGCGGAGGAAAAG	No	Sandhu et al., 1995
P2	CTCTGCTTCACCCTATTC		Sandhu et al., 1995
U1	GTGAAATTGTTGAAAGGGAA	No	Sandhu et al., 1995
U2	GACTCCTTGGTCCGTGTT		Sandhu et al., 1995
ITS1-F	CTTGGTCATTTAGAGGAAGTAA		Gardes and Bruns, 1993
TW14	GCTATCCTGAGGGAAACTTC		Taylor and Bruns, 1999
Ctb6	GCATATCAATAAGCGGAGG		Taylor and Bruns, 1999
TW13	GGTCCGTGTTTCAAGACG		Taylor and Bruns, 1999
	ITS-5.8S RNA Gene		
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	Basidiomycete[d]	Baar et al., 1999
ITS4-B	CAGGAGACTTGTACACGGTCCAG		Gardes and Bruns, 1993
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	Ascomycete	Baar et al., 1999

Table 27.6 Sequence of Primer Pairs Used in Environmental Studies and Their Fungal Specificity (Continued)

Primer	Sequence	Fungal Specificity	Reference
ITS4-A	CGCCGTTACTGGGGCAATCCCTG		Larena et al., 1999
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	NT	Baar et al., 1999
ITS4[a]	TCCTCCGCTTATTGATATGC		White et al., 1990
EF4	GGAAGGGRTGTATTTATTAG	Possibly not	Smit et al., 1999
ITS4[a]	TCCTCCGCTTATTGATATGC		White et al., 1990
ITS1-F-GC	CTTGGTCATTTAGAGGAAGTAA	NT	White et al., 1990
ITS2[a]	GCTCCGTTCTTCATCGATGC		White et al., 1990

Note: -GC = The prefix or suffix -GC with the name of a primer refers to the presence of a GC clamp attached to the primer. The sequence of the GC clamp used varies between reports. NT = Not tested.

[a] NS primers described by White et al. (1990) are not fungal specific.
[b] Primer D is primer 1860 described by Elwood et al. (1985).
[c] Not tested with animal sequences directly.
[d] See Horton and Bruns (2001) for comments on specificity.
[e] See Anderson et al. (2003b) for comments on specificity and bias of nu-ssu-0817-5′, nu-ssu-1196-3′, and nu-ssu-1536-3′.
[f] Zuccaro, unpublished observation.
[g] See Borneman and Hartin (2000) for specificity of EF3, EF4, and fung5.
[h] See Zuccaro et al. (2003) for specificity of NS1-FR1GC.

because they might be heterogeneous (Buchholz-Cleven et al., 1997; Vallaeys et al., 1997; Jackson et al., 2001). Regions from the 18S, 28S, and ITS-5.8S rDNA have been used in DGGE and TGGE analysis to determine the community structure of fungal populations (Table 27.7). The sizes of PCR fragments separated in these studies varied from 230 to 1600 bp. DGGE and TGGE analyses are usually performed with short DNA fragments to avoid problems associated with multiple or diffuse band formation (Kisand and Wilkner, 2003); however, several reports (Vainio and Hantula, 2000; Gomes et al., 2003) observed good band separation with larger fragments. This separation, however, may be due to length heterogeneities of the nuSSU rDNA fragments amplified (Zuccaro et al., 2003).

DGGE and T-RFLP techniques have been used to fingerprint fungi directly from the environment in order to determine community structure (Smit et al., 1999; Buchan et al., 2002; Dickie et al., 2002; Klamer et al., 2002; Brodie et al., 2003; Gomes et al., 2003; Nikolcheva et al., 2003). Only two studies (Brodie et al., 2003; Nikolcheva et al., 2003) made direct comparisons between the two techniques in terms of the number of bands detected, but reported conflicting results. Brodie et al. (2003) reported greater detection with T-RFLP, whereas Nikolcheva et al. (2003) observed more variation with DGGE. Both reports, however, compared the techniques using different primer systems, so that any variation between the methods may reflect primer bias. Other assessments of fungal diversity and community structure have been made after the cloning of environmental amplification products followed by RFLP and sequencing analyses (Horton and Bruns, 2001; Vandenkoornhuyse et al., 2002; Landeweert et al., 2003). The few studies undertaken suggest that molecular fungal diversity may be extensive, but also highlight a problem of sequence identity resolution (Kowalchuk et al., 1997; van Elas et al., 2000). The reliance upon nuSSU rRNA gene sequences, which resolve little beyond the order/family taxonomic level, or ITS-5.8S rRNA regions that provide clone identification but limited higher-order placement (Bruns et al., 1998; Horton and Bruns, 2001; Anderson et al., 2003a) complicates the identification of unknown or cryptic fungi. Sample inconsistencies in sequence and T-RFLP databases for taxa and genes, and incorrectly submitted taxon information, further exasperate the situation (van Elas et al., 2000; Bridge et al., 2003), making the creation of a culture database important for the further identification of retrieved sequences.

The genetic typing of strains is traditionally achieved by RFLP and T-RFLP analyses or by ARDRA using the ITS-5.8S rDNA region (Taylor and Bruns, 1999; Osborn et al., 2000; Klamer et al., 2002; Gomes et al., 2003). DGGE or TGGE can also be used to type fungal strains, but without resorting to restriction analysis (Hernán-Gómez et al., 2000). DGGE and TGGE separate molecules according to their lowest region, or domain, of thermostability during DNA strand disassociation (Uitterlinden and Vijig, 1994). The genetic typing is therefore based on the sequence in the first domain to be denatured or melted. Molecules with identical melting domains will migrate to the same position within the gel after electrophoresis, providing a basis for the initial matching of sequences between isolates and environmentally retrieved fragments. It should be pointed out, however, that different sequences might share the same band position after electrophoresis if the lowest melting domains are similar (Sekiguchi et al., 2001). Further sequence characterization of DGGE/TGGE bands is therefore essential to identify any relationship between the environmental and isolate sequences. Alignment and phylogenetic analyses of sequences from environmental fragments and isolates provide a framework to (1) ascertain the taxonomic position of both the environmental unknown and the matching isolate, (2) identify novel lineages within known groups, and (3) confirm the identity provided after BLAST searches. This approach is important not only for dealing with environmental sequences exhibiting low homology matches, but also for confirming BLAST identities, as these may be incorrect (Bridge et al., 2003).

Table 27.7 Primer Pairs and Molecular Separation Used to Analyze Environmental DNA

Primers	Separation Method and Conditions	Product Separated bp	Reference
	nuSSU rRNA Gene		
Nested: (1) NS1-NS8 (2) NS1GC-NS2+10	DGGE: 8% w/v acrylamide; 25–45% denaturant; 16 h at 85 V at 60°C	~400	Kowalchuk et al., 1997
Nested: (1) EF4f-EF3r (2) NS2f-fung5GC	DGGE: 6% w/v acrylamide; 35–65% denaturant; 12 h at 100 V at 60°C	230	van Elas et al., 2001
Nested: (1) EF4-EF3 or EF4-fung5 (2) EF4-NS3-GC	TGGE: 8% w/v acrylamide; 36–44°C; 17 h at 110 V	~350	Smit et al., 1999
NS1-FR1GC	DGGE: 7.5% acrylamide; 18–43% and 18–38% denaturant; 17 h at 180 V at 58°C	~1600	Vainio and Hantula, 2000; Pennanen et al., 2001
EF390-FR1GC	DGGE: 7.5% acrylamide; 45–60% denaturant; 18 h at 50 V at 58°C	347	Vainio and Hantula, 2000; Pennanen et al., 2001
NS26-518GC	DGGE: 10% acrylamide; 20–45% denaturant; 3.5 h at 200 V at 60°C	316	Schabereiter-Gurtner et al., 2001
EF4-518GC	DGGE: 10% acrylamide; 20–45% denaturant; 3.5 h at 200 V at 60°C	426	Schabereiter-Gurtner et al., 2001
Nested: (1) EF4-EF3 (2) EF4-NS3GC	DGGE: 10% acrylamide; 30–45% denaturant; 17 h at 85 V at 60°C	~500	Brodie et al., 2003
YUNIV1-GC- YUNIV3	TGGE: 8% acrylamide; 62–65°C; 3 h at 130 V	244	Hernán-Gómez et al., 2000
nu-SSU-0817–nu-SSU-1536	T-RFLP		Brodie et al., 2003

nu-SSU-0817–nu-SSU-1196	Molecular cloning	420	Anderson et al., 2003b
nu-SSU-0817–nu-SSU-1536	Molecular cloning	760	Anderson et al., 2003b
EF4-EF3	Molecular cloning	1500	Anderson et al., 2003b
NS1-FR1	DGGE: Acrylamide; 18–43% denaturant; 18 h at 180 V at 58°C	~1600	Gomes et al., 2003
NS1-GCfung	DGGE: 8% acylamide; 20–55% denaturant; 12 h at 70 V at 56°C	370	Nikolcheva et al., 2003
F1300-D	T-RFLP		Nikolcheva et al., 2003
NS5-D	T-RFLP	680	Nikolcheva et al., 2003
NS1-GCfung	DGGE: 7% acrylamide; 15–50% dentaturant; 20 h at 50 V at 60°C	350	May et al., 2001
Nested: NS1-FR1 NS1-EF3GC	DGGE: 7.5% acrylamide; 18–45% denaturant; 16 h at 180 V at 58°C	~1650	Zuccaro et al., 2003
AU2-AU4	Molecular cloning		Vandenkoornhuyse et al., 2002
	nuLSU rRNA Gene		
Nested: (1) ITS1-F–TW14 (2) Ctb6-TW13	Molecular cloning	~650	Taylor and Bruns, 1999
Nested: NL209-NL912 NL359-NL912GC	DGGE: 7.5% acrylamide; 38–60% denaturant; 18 h at 60 V at 58°C	559	Zuccaro et al., 2003
Nested: (1) P1-P2 (2) U1GC-U2	DGGE: 10% acrylamide; 35–65% denaturant; 6 h at 150 V at 60°C	260	Möhlenhoff et al., 2001
	ITS-5.8S rDNA Region		
ITS1-F–ITS4	T-RFLP		Dickie et al., 2002

Table 27.7 Primer Pairs and Molecular Separation Used to Analyze Environmental DNA (Continued)

Primers	Separation Method and Conditions	Product Separated bp	Reference
ITS1-F–ITS4 ITS1-F–ITS4-B ITS1-F–ITS4-A	T-RFLP		Klamer et al., 2002
ITS1-F–ITS4	T-RFLP		Dickie et al., 2002
ITS1-F–ITS4	Molecular cloning	450–750	Anderson et al., 2003b
Nested: EF3-ITS4 ITS1-F(GC)–ITS2	DGGE: 8% acrylamide; 10–50% denaturant gradient; 16 h at 75 V at 60˚C	300, but range of 400–600 reported	Anderson et al., 2003a

27.3.3.3 Molecular Strategy to Assess the Diversity of Fungi Associated with Fucus serratus

The molecular identification of the principal fungal populations associated with *Fucus* would provide a baseline to follow epiphytic, endophytic, disease-causing, and mutualistic associations. In developing a strategy to assess the molecular diversity of fungi associated with seaweeds, a number of factors have to be taken into account: The fungal community is likely to be of low density, comprising a small number of active members among nongrowing propagules. Fungi may be associated with epiphytes and other organisms present in the canopy, rather than with the algae itself. The seaweed thalli are also likely to contain compounds that inhibit microbial growth, and these might interfere with PCRs. An additional problem is the poorly subscribed sequence database for marine algicolous fungi, which complicates the molecular identification. For all these reasons, efficient fungal-specific primers, an extraction procedure that yields DNA free from environmental contaminants, the availability of a database comprising cultured isolates from the area of study, and a means to compare environmental sequences with those from cultured isolates are necessary.

Ascomycetes represent the main group of fungi associated with seaweeds, but their DNA only constitutes a minor fraction of that extracted from algal thalli. For this reason, the strategy adopted to determine the molecular diversity had to incorporate a system for detecting rare ascomycete sequences that could be matched to culture isolates. Two PCR primers systems were employed, one generic and one that was ascomycete specific, in conjunction with DGGE and molecular cloning to separate individual molecules for sequence comparison. An integrated sampling regime was incorporated within this scheme where isolates were cultured from the same sample material used to extract DNA. The novel nuLSU primers (NL204, NL359, and NL912) were designed to preferentially amplify ascomycete sequences, whereas the nuSSU primers (NS1, FR1, EF3) amplified fungal and animal sequences (Zuccaro et al., 2003). The use of two different primer systems permitted a comparison to be made between molecular diversity assessments. DNA extracted from algal thalli was purified using cesium chloride gradient density centrifugation and fungal sequences amplified using a nested approach (Table 27.7). Sequences were cloned after the first amplification to provide a comparison between the reactions. Fragments generated after the second reaction were subjected to DGGE, which could be compared with the band migrations of cultured isolates. The majority of retrieved bands from the 18S-DGGE gels were cloned and sequenced, and all of the bands from the 28S-DGGE gels were cloned and sequenced or sequenced directly from excised bands, including those from isolates that exhibited similar migration patterns (Zuccaro et al., 2003). The sequences recovered from algal thalli and cultured isolates were subjected to BLAST searches for initial identification before further characterization. Exact sequence homologies were calculated from alignments of related taxa, providing an accurate sequence match, and the evolutionary relationships of the environmental sequences were determined after phylogenetic analysis.

27.3.3.4 Molecular Diversity of Fungi Associated with the Fucus serratus Canopy

PCR-DGGE analysis of DNA extracted from water-treated or surface-sterilized healthy fronds and casts using primers for the nuSSU and ascomycete nuLSU rRNA genes revealed a mixture of sequences representing 12 ascomycete phylotypes (Zuccaro et al., 2003, unpublished data). The retrieved nuSSU sequences provided sequence homology matches

to general groups with equal similarities for a number of related taxa, whereas the nuLSU sequences gave more exact identities to isolates. The sequences of the phylotypes represented four orders: the Dothideales, Halosphaeriales, Hypocreales, and Lulworthiales (Figure 27.2c). Only four phylotypes could be unambiguously matched to the sequences of isolates recovered from *F. serratus*: two matches within the Halosphaeriales (*Corollospora angusta* and *Sigmoidea marina*), one within the Lulworthiales (*Lindra* cf. *obtusa*), and one for a series of hypocrealean isolates (an *Emericellopsis*/*Acremonium* group). The identities of the remaining phylotypes fell broadly within the Dothideales, Hypocreales, and Lulworthiales after BLAST searches and phylogenetic analysis.

The most common sequence recovered from all of the samples matched that from *Sigmoidea marina* (Haythorn et al., 1980). In a phylogenetic analysis, this sequence grouped within the genus *Corollospora*, forming a strongly supported clade with *Corollospora angusta*, another sequence that was retrieved from *F. serratus* (Figure 27.3). The recovery of these sequences was not equal in frequency, with that of *C. angusta* being retrieved mainly from casts. In contrast, the sequence for *S. marina* was routinely isolated from healthy fronds throughout the year. Both of these are obligate marine fungi, and *S. marina* has been implicated in the decomposition of algal casts (Haythorn et al., 1980; Nakagiri and Tokura, 1987). The sequences of two other phylotypes grouped within the Lulworthiales, an exclusively obligate marine clade, as a unique lineage after phylogenetic analysis (Figure 27.4). In a subsequent analysis the sequence from the *Fucus* isolate *Lindra* cf. *obtusa* matched one of these phylotypes and migrated to an identical gel position after DGGE.

Corollospora angusta and *Lindra obtusa* are considered to be arenicolous fungi, attaching to sand and calcareous shell particles on the beach, although they were originally isolated from sea foam (Nakagiri and Tokura, 1987). Calcareous tubes formed by worms were not removed from the thalli of *F. serratus*, and it is possible that these formed an attachment point prior to hyphal extension into algal tissue (Zuccaro et al., 2003). Spores from arenicolous fungi, such as *C. angusta* and *L. obtusa*, can germinate on silica and calcareous material within a few hours and possibly grow into necrotic algal tissue, exploiting the seaweed (J. Kohlmeyer and B. Volkmann-Kohlmeyer, personal communication).

Three hypocrealean isolates, *Emericellopsis minima*, *Acremonium* species 264, and *Acremonium tubakii* retrieved from *F. serratus*, matched one environmental DGGE band. Representatives of *Emericellopsis* commonly share an *Acremonium* conidial stage, explaining why the nuLSU rDNA sequences from these isolates were similar, making it difficult to distinguish between them molecularly (Zuccaro et al., 2003). The sequence analysis therefore failed to identify which isolate was active within the algal thalli. One candidate for this match is *Acremonium fuci*, which was routinely isolated from living, including surface-sterilized tissues, and dead fronds. The other isolates were recovered sporadically and in this respect did not match the routine detection of the *Emericellopsis*-like sequence after DGGE analysis. The comparison of molecular and cultured diversities provides an alternative means of assessing the isolates recovered from fronds, illustrating the importance of combining different techniques and approaches. In general, few sequences from isolates matched those from the environment, indicating that colonization for most was limited.

One phylotype detected from *F. serratus* matched, with a low homology of 96%, a nuLSU rRNA gene sequence from a species of *Engydontium*, a member of the Clavicipitaceae. This family comprises pathogens of grasses, arthropods, and fungi. The unidentified clavicipitacean is, presumably, not growing in direct association with *F. serratus*, but with an arthropod inhabitant of the algal canopy. A variety of animal sequences were recovered after SSU rDNA-DGGE analysis, including those from annelids, hydroids, and

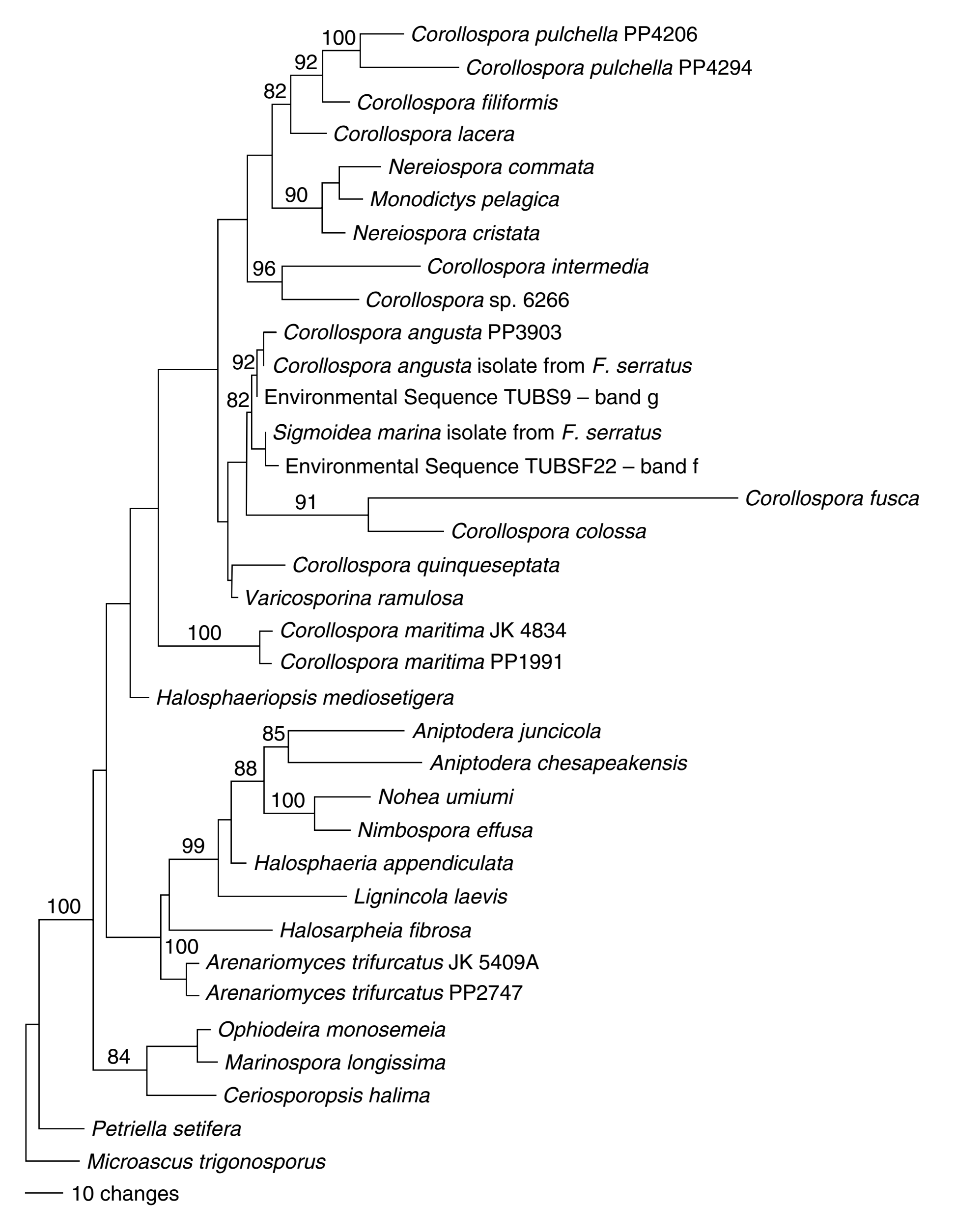


Figure 27.3 Relationship of *C. angusta*, *S. marina*, and environmental sequences recovered from *F. serratus*. Maximum parsimony analysis generated three trees of 1748 steps (CI = 0.609, RI = 0.501, RC = 0.305, HI = 0.391) from 375 parsimony informative characters (pics). Bootstrap values over 80% are shown above the branches. CI = consistency index; RI = retention index; RC = rescaled consistency index; HI = homoplasty index. (From Zuccaro et al., *Mycol. Res.,* 107:1451–1466.)

arthropods. These sequences may represent eggs deposited on the algal surface, as no adult or larval forms, except for calcareous worm tubes, were observed on the thalli after cleaning (Zuccaro et al., 2003). The presence of one other phylotype belonging to the Hypocreales was recorded. This matched sequences from the genus *Hypocrea* after a BLAST search, but it was observed infrequently.

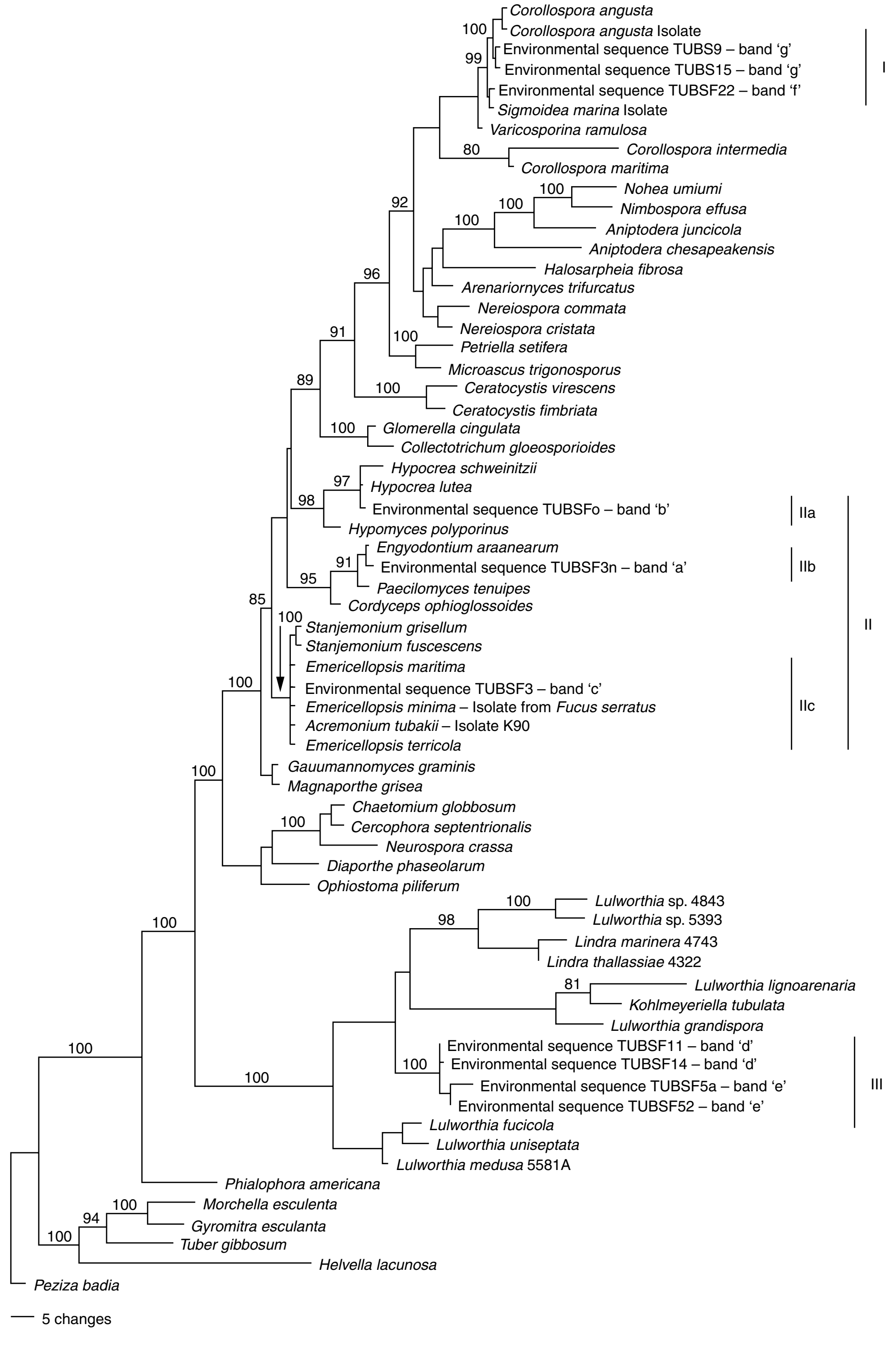


Figure 27.4 Phylogram showing the relationships of the 28S rRNA gene sequences recovered from *F. serratus*. Maximum parsimony analysis generated the six most parsimonious trees of 2892 steps (CI = 0462, RI = 0.655, RC = 0.303, HI = 0.538) from 555 pics. Comparison of the log likelihood for each tree using the Kishino–Hasegawa test revealed that the tree depicted had the lowest –InL (17895.12021) at p = 0.0001, 0.01, and 0.65. CI = consistency index; RI = retention index; RC = rescaled consistency index; HI = homoplasty index.

Three nuLSU and one nuSSU phylotype recovered after cloning gave low homology matches to species of the Pleosporales. The exact identities of these sequences have not been resolved. These sequences were recovered from casts only, in agreement with the culture isolation study where a large number of species from the Dothideales were isolated (Figure 27.2a and b). This suggests that these species might be more active in the decomposition of algal casts. Interestingly, no sequence match was retrieved for *Dendryphiella salina*, the most frequently isolated member of the Dothideales, despite successful PCR amplification of the isolate DNA using both primer sets. The DGGE separation of these bitunicate nuLSU rDNA cloned sequences was poor, with the bands producing smears characteristic of molecules with multiple melting domains (Kisand and Wilkner, 2003) under the standardized denaturing conditions used (Zuccaro et al., 2003). The presence of unresolved bands has been reported in other studies using DGGE (Brodie et al., 2003), making additional assessments, such as the molecular cloning of sequences directly from environmentally amplified fragments, necessary when using this technique.

The DGGE profiles of fungal fragments amplified from individual algal thalli documented the prevalent populations associated with the algal canopy. Comparison of these profiles between thalli revealed that there was a low species complexity for both genetic systems used. The nuSSU system amplified animal sequences that often comigrated with fungal fragments, making direct comparisons difficult. The nuLSU system, however, only amplified fungal sequences, and the DGGE profiles for these fragments varied from one to five bands between individual thalli. This low molecular diversity might be an underestimate, as a result of undetected PCR or sampling bias, but it suggests that fungal colonization of *F. serratus* thalli is limited. Bands corresponding to *Sigmoidea marina*, the *Emericellopsis*/*Acremonium* group, and *Lindra obtusa* were routinely observed amplified from healthy and decaying thalli, suggesting that populations of these fungi are active in the *Fucus* canopy. The exact relationship of each species is unknown. *S. marina* and *L. obtusa* are known as saprobes associated with decaying algal and other materials. The presence of these organisms molecularly from casts corresponds with their saprophytic nature; however, their presence with healthy fronds suggests that the association with *F. serratus* might be more complicated, with colonization occurring earlier when the thalli are still attached. Species of *Emericellopsis* and related *Acremonium* isolates, generally known as soil isolates (Domsch et al., 1980), have been reported as potential endophytes in aquatic environments (Shearer, 2001). Some species have been recorded previously from marine environments, primarily mud-derived ones, leading to suggestions that these might be active in coastal systems (Tubaki, 1973). The routine detection of an *Emericellopsis*-like sequence associated with the canopy of *F. serratus* indicates that this may be true.

27.4 NUTRIENT EXPLOITATION AND FUNGAL DISTRIBUTION IN ALGAL THALLI

27.4.1 Fungal Exploitation of Algal Nutrients

Highly water soluble polyols (or sugar alcohols) are widely distributed among algae and fungi and contribute significantly to their biomass. These compounds are considered to be physiologically essential, acting as carbohydrate reserves, translocatory compounds, and reservoirs of reducing power, and function as osmo- and coenzyme regulators (Jennings, 1984). Furthermore, they are involved in the maintenance of chemical potential gradients for carbon movement in host–parasite and mutualistic relationships (Jennings, 1984; Pfyffer, 1998; Gimmler, 2001). In lichens with green algal photobionts, the acyclic polyols ribitol, erythritol, and sorbitol have been identified as mobile carbohydrates moving from the autotrophic to the heterotrophic partner (reviewed by Honegger, 1997).

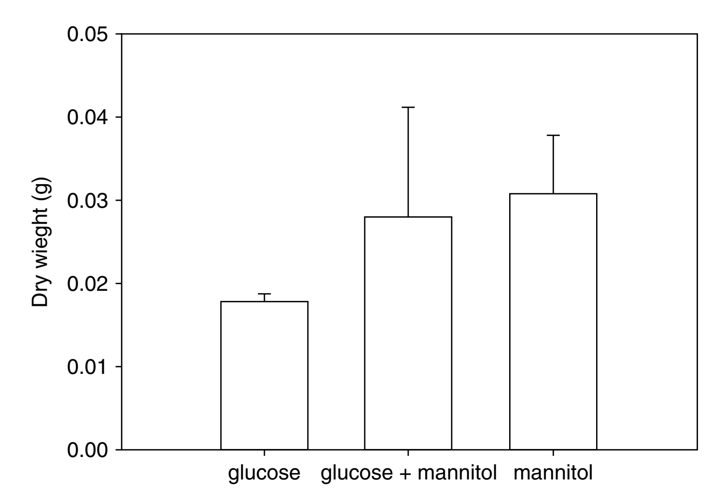


Figure 27.5 Growth of *S. marina* on different liquid media. This fungus grew significantly better (one-way ANOVA, $p = 0.036$) with mannitol (0.1 g/l) than with glucose (0.25 g/l) as the only C source.

Glycerol is the main osmotic solute produced by *Dunaliella acidophila* in association with species of *Bispora*. Concentrations of this solute may reach levels up to 3 m*M* in the culture medium, with *Bispora* capable of using it as a carbon source (Fuggi et al., 1988; Gimmler and Weis, 1992; Gimmler, 2001). *Fucus*-inhabiting fungi may behave in a similar manner, using the osmotic solute produced by the algae as a carbon source and accumulating it within their cells. The main sugar alcohol detected in water and water–ethanol extracts of *Fucus serratus* is mannitol (Zuccaro et al., 2001), and it is the primary substance to be translocated within the thallus, together with amino and organic acids of low molecular weight (Diouris, 1989). The metabolic turnover of glycerol and mannitol is very rapid in fungi (Holligan and Jennings, 1972; Dijkema et al., 1985; Wethered et al., 1985), leading to the suggestion by Jennings (1990) that polyols may contribute to energy flow during the growth of marine fungi, particularly under saline stress or nutrient-limiting conditions, as well as function as compatible osmolites. Mannitol is the main storage compound detected from water–ethanol extracts from *Sigmoidea marina*, a marine hyphomycete isolated from *F. serratus*, together with trehalose and glucose (Zuccaro, unpublished, using the extraction method described by Wagner, 2002). The growth of this fungus was best on mannitol, compared with glucose (one-way ANOVA, $p = 0.036$), in the presence of salt (Figure 27.5), although the concentration of mannitol in the mycelium remained relatively constant irrespective of the carbon source (mannitol or glucose) used. The maintenance of intracellular mannitol levels in *S. marina* indicates that this osmotic solute may be important for reasons other than those expected from general metabolism.

Polyols are not the only nutrients involved in fungal–algal interactions. Proteins, neutral sugars, and complex polysaccharides may also be implicated in fungal colonization. The dry weight of *Fucus vesiculous*, a closely related species of *F. serratus* (Leclerc et al., 1998), comprises 7% w/w protein and 2.5% w/w lipid with neutral sugars, mainly glucose and fructose, but also arabinose, xylose, mannose, and galactose representing 8% w/w (Rupérez and Saura-Calixto, 2001). The cell wall of *Fucus* species also contains complex polysaccharides such as cellulose, fucoidan, and alginic acid, as well as tannins that are potential substrates for fungi during colonization. In an attempt to identify patterns of substrate utilization in fungi isolated from *F. serratus*, the enzyme activities of the

predominant isolates were examined. All the fungi examined grew better on agar media containing salt. Amylase, cellulase, and particularly protease activities were detected in all isolates tested. No activity was recorded for β-glucosidase, with the exception of *Lindra obtusa*, and polyphenoloxidase (Table 27.8a). Laminarinase activity has been reported for *Dendryphiella salina*, *Dendryphiella arenaria*, *Lindra thalassiae*, and *Varicosporina ramulosa*, all of which are algicolous (Tubaki, 1969). The isolates recovered from *F. serratus* could also all use laminarin as a sole carbon source (Table 27.8b); furthermore, *Acremonium fuci* isolate T.U.B. 264 could use fucose with an uptake ratio similar to that for glucose (Zuccaro et al., 2005). Schaumann et al. (1986) recorded laminarinase activity from 71% of the *Lulworthia* isolates collected from driftwood, suggesting that this enzyme might be common among marine fungi.

27.4.2 Fungal Vertical Distribution

The distribution of fungal species in a host may change quantitatively and qualitatively according to the age, tissue, anatomy, or physical state of the substrate sampled. In *Spartina alterniflora*, a halophyte of salt marshes, the fungal community changes according to which parts of the plant are submerged (Kohlmeyer and Kohlmeyer, 1979). Factors that influence moisture levels, chemical composition, temperature, metabolite availability, and other variables may generate niche differentiation across vertical gradients (Malajkzuk and Hingston, 1981). Molecular analyses of fungal community structure associated with *Phragmites australis* (Wirsel et al., 2001) and *Pinus resinosa* (Dickie et al., 2002) demonstrate clear examples of such differentiation in aquatic and terrestrial environments. Potential vertical distributions of fungal populations associated with seaweeds remain largely unexplored. A number of algicolous fungi exhibit some localization during infection, such as the infections of *Lautitia danica* and *Lindra crassa*, or display differential hyphal densities in algal thalli, as in the endophytic associaton of *Mycophycias ascophylli* (Section 27.2.1). In order to detect any potential localization in fungal species distribution, the growing tips, receptacles, blade, and foot regions were dissected from thalli of *Fucus serratus* prior to the molecular and culturing analyses (Section 27.3.1).

Isolates were recovered from all of the algal body parts, but fewer were cultured from the growing tips and receptacles than from the blade tissues. This pattern was also observed molecularly. The DGGE profiles from receptacle and apical tip tissues contained fewer gel bands than those observed from the rest of the body parts. A number of reasons might explain the limited fungal colonization observed in the meristematic and receptacle regions. In younger tissues, fungi have less time for development, reducing the number of colonizing species and their overall biomass. Differences in nutrient availability and metabolite production within the thallus might also influence fungal colonization patterns. Certainly, the production of bioactive compounds appears to differ in these specialized regions, with a greater activity observed against invasive organisms in the younger tissues than in the mature thallus (Section 27.2.2). Brenchley et al. (1997) reported that in *F. serratus* the photosynthetic capacity of the receptacle tissue was approximately 50% lower than that of the vegetative thallus. The concentration of mannitol, a primary product of photosynthesis, is lower in water–ethanol extracts from growing tips and receptacles than in that found in the holdfast and blade (Zuccaro and Wagner, unpublished data). Metabolite concentration gradients present in the algal thallus suggest the movement of nutrients, with growing areas and reproductive structures acting as resource sinks (Lobban and Harrison, 1994). The effect on fungal infection and colonization of such gradients could possibly lead to a vertical distribution. The release of nutrients into the environment from senescent cells present in the algal thallus could also influence the site of fungal infection. These cells are likely to be present in the mature parts of the thallus, rather than in the

Table 27.8 Enzyme Profiles and Carbohydrate Utilization of Selected Fungi Isolated from *Fucus serratus*

***Fucus serratus* Isolates**[a]	**Cellulase**	**β-Glucosidase**	**Amylase**	**Lypase**	**Protease**	**Poliphenoloxidase**
Acremonium fuci T.U.B. 264	++	–	+	+	+	–
Sigmoidea marina	+	–	+	–	+	–
Corollospora angusta	+	–	+	+	++	–
Lindra cf. Obtusa	++	+	nt	–	++	–
***Fucus serratus* Isolates**[b]	**Glucose**	**Fucose**	**Laminarin**	**Fucoidan**		
Acremonium fuci T.U.B. sp. 264	++	+	++	–		
Sigmoidea marina	+	–	+	–		
Corollospora angusta	+	–	+	–		
Lindra cf. Obtusa	++	–	++	–		

Note: nt: not tested.

[a] The enzymes test was carried out on agar plates containing 33 g/l salt. Three parallels were inoculated for each variants and stored in the dark at 20°C.

[b] Utilization of different algal carbon sources: D-(+)-glucose, L-(–)-fucose, laminarin from *Laminaria digitata* (Fulka) and fucoidan from *Fucus vesiculosus* (Fulka).

younger growing regions, and would provide areas where fungal colonization might be more favorable.

The only unambiguously matched sequence amplified from all the different body parts corresponded to that of the *Sigmoidea marina* isolate in agreement with the cultural diversity (see also Section 27.3.2). A sequence representing the *Emericellopsis/Acremonium* group, possibly *Acremonium fuci*, was routinely detected from the receptacle tissues; however, other tissues yielded this strain culturally as well as molecularly, suggesting that its presence may be due to persistent colonization rather than specialization. No authentic vertical distribution for fungal colonization, where particular species dominate specific tissues, was observed molecularly or culturally. This may reflect the fact that the different tissues of *F. serratus*, although relatively differentiated compared with other classes of algae, are fairly homogeneous with similar structures and functions throughout the thallus. Areas for potential infection would therefore be structurally similar and could be subjected to random saprophytic colonization events, particularly if they are damaged or dead.

27.5 CONCLUSIONS

In mycology the adoption of molecular techniques that describe community structure and function is relatively recent. The application of these techniques in diversity studies is reliant upon the availability of taxonomically broad-based sequence databases from which identifications can be made. For most environments, this extensive resource is not available or is inadequate. The incorporation of culturing techniques and other traditional methods with molecular ones in an integrated or combined approach provides one way to generate a database for sequence comparison. The importance of molecular systematics and taxonomy of fungi in molecular ecological studies should also not be overlooked. Phylogenetics studies provide a basis for determining the correct genetic region to use in an environmental analysis and a means of assessing the identity of novel lineages.

The inclusion of a cultured database proved indispensable in describing the community structure associated with *Fucus serratus* molecularly, allowing environmental sequence matches to known isolates and identifying sequences that represent uncultured fungi. This approach also provided a basis to better understand the molecular profiles generated after PCR-DGGE analysis. The identity of *Sigmoidea marina*, for which no sequence exists in databases, within the canopy community structure and its relationship with the genus *Corollospora* would not have been possible using the environmental molecular data and BLAST searches alone. Broad fungal taxonomic groups do not necessarily represent physiological or biochemically related assemblages that might be more ecologically significant. A molecular profile for an environment, or substrate, detailing just broad taxonomical groupings can therefore be considered as only a preliminary description. Moving beyond this level requires a more integrated approach targeting specific groups and perhaps different genetic loci.

The identity and extent of lineages representing novel or nonculturable organisms in the environment are important aspects of fungal molecular ecology studies. An accurate assessment of such groups is reliant, however, on the state of fungal molecular systematics. Within the *F. serratus* canopy and casts, a number of sequences representing novel lineages within the Hypocreales, Dothideales, and Lulworthiales were originally observed after phylogenetic analysis. The molecular systematics of these orders is far from complete, though, and it is conceivable that many of these unidentified lineages represent organisms known morphologically but not molecularly. This was certainly true for one group of

environmental sequences located within the Lulworthiales, which was eventually matched to *Lindra obtusa*.

The availability of cultures that can be linked to community profiles creates a basis for further research. The detection of an *Emericellopsis*-like sequence from the canopy of *F. serratus* provided evidence that these organisms are active in the marine environment, but it was the accessibility of isolates that allowed further physiological and phylogenetic studies. These are in progress at present, providing more information on the molecular systematics of groups detected in the seaweed canopy and generating evidence for the types of associations involved. In this respect, the molecular analysis provided a framework to reevaluate the nature of fungal communities associated with seaweeds and the impetus to extend research using environmentally matched isolates.

The fungal communities detected molecularly in the canopy of *F. serratus* represent a limited range of ascomycetes, in broad taxonomic agreement with the cultured diversity. The extent of the diversity is greater than expected, however, with a number of phylotypes unmatched to isolates. The majority of the fungi detected were mainly known to be saprophytic, with no obvious parasitic forms recorded. No sequence information exists on the algicolous parasites, so that their molecular identification with environmentally amplified fragments is difficult. The presence of active nonassociated fungi and saprophytes attached to healthy fronds further complicates the molecular identification of these parasites. The detection of novel phylogenetic lineages might represent parasitic forms; however, additional conformation is required with the development of specific hybridization probes for *in situ* detection and direct microscopic observation. The molecular approaches described to assess the fungal diversity of *F. serratus* provide strategies to examine other fungal–algal associations and a means to the development of probes for the detection of nonculturable and parasitic fungi.

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28

Trophic Interactions of Fungi and Animals

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28.1 INTRODUCTION

Fungi are an important food resource in soil. Fungal tissue is composed of chitin, about 14% N, and reproductive structures such as sclerotia and sporocarps containing high P concentrations. Fungal biomass may constitute as much as 10% of soil and litter (Dighton, 2003). Fungal structures include hyphae, spores, mycelial cords, ectomycorrhizal root tips, and sclerotia. Hyphae are differentiated into runner hyphae, absorptive hyphal networks, and hyphal bridges (Friese and Allen, 1991). All these structures can be used by the soil fauna as food resources. The way they are spread throughout the soil, for example, diffusely or in patches, affects feeding strategies of the fungivore fauna (Bengtsson et al., 1993).

Fungi are able to grow into substrates to obtain and transport nutrients and form associations with roots. Mycelial cords and rhizomorphs act as organs for bulk transport of carbon and nutrients across nutrient-free environments (Deacon, 1997). Fungi therefore are an important factor for energy flux through soil systems and constitute a fungal energy channel in the detrital food web (Moore et al., 1988; Scheu and Setälä, 2002). Besides this, fungi control decomposition processes due to their superior enzymatic capabilities (Dix and Webster, 1995; Rosenbrock et al., 1995; Frankland, 1998) and ability to translocate nutrients. The detritivore soil fauna may shred plant residues, but it is not capable of breaking down recalcitrant compounds in organic matter. The carbon fixed in plant biomass becomes available to the detritivores by recycling via fungi, with a degradation potential for lignin or humic acids.

28.2 FEEDING BEHAVIOR

The basic chelicerate mouthparts of mites, the mandibular mouthparts of collembola, diplura, and protura, and the stylets of nematodes have been modified for feeding methods such as cutting, grinding, scraping, piercing, and sucking. This means that fungal structures can be ingested in a variety of ways. Hyphae may be cut off and ingested like spaghetti (oribatid mites; J.L., personal observation) or may be penetrated by a stylet or modified mandibles and hyphal contents sucked out (nematodes, Yeates et al., 1993; protura, Sturm, 1959; collembola, Rusek, 1998). Spores may be ingested whole (oribatid mites, collembola; Lussenhop and Wicklow, 1984), or opened and their contents eaten (probably prostigmatid mites; Gochenaur, 1987). The rind of sclerotia may be scraped and the contents consumed (mycetophilid fly larvae; Anas and Reeleder, 1988).

On the soil surface and above, mushrooms, shelf fungi, and other fungal structures are fed on by beetles, flies (Courtney et al., 1990), and vertebrates. In one case, at least, fungivory by ciid beetles reduced the fitness of the host fungus, *Coriolus versicolor* (Guevara et al., 2000). Consumption of sporocarps by vertebrates is opportunistic, but is important in dispersing spores (Johnson, 1996), even those of arbuscular mycorrhizal fungi (Mangan and Adler, 2002).

28.3 FUNGAL GRAZING SOIL FAUNA

Fungivory is widespread among soil invertebrates and also among mammals. Soil invertebrates are often divided into microfauna (protozoa, nematodes), mesofauna (mites, collembola, protura), and macrofauna (enchytraeids, earthworms, millipedes, centipedes). Generally, protozoa are regarded as bacterial feeders, but some species of naked amoebae, testate amoebae, ciliates, and flagellates are obligate fungivores (Foissner, 1987; Hekman et al., 1992; Ekelund and Rønn, 1994). Several large amoebae are able to feed on conidial spores and fungal mycelium, whereby fungivory occurs in many different and not closely related genera (Ekelund and Rønn, 1994). Experiments from Heal (1963) showed that yeast can also be a suitable food source for bacterial-feeding amoebae.

Nematodes are the majority of the fungal-feeding microfauna. They are known to cause severe damage to fungal cultures *in vitro*. They may destroy aerial hyphae and limit fungal growth (Mankau and Mankau, 1963; Riffle, 1967; Shafer et al., 1981; Rössner and Nagel, 1984; Ruess and Dighton, 1996). Most important is their grazing on mycorrhizal fungi, which may restrict mycorrhizal development and limit nutrient uptake by plants (Clark, 1964; Riffle, 1975; Giannakis and Sanders, 1987, 1989). This can lead to a reduction in the yield of mycorrhizal host plants (e.g., trees) or the crop of cultivated mushrooms (Arrold and Blake, 1968; Giannakis and Sanders, 1989).

Among the soil mesofauna, protura are predominantly fungal-feeding, in particular on ectomycorrhiza (Sturm, 1959). They are known to prefer freshly mycorrhized root tips, but feed also on fungal hyphae growing through the soil (Nosek, 1975). There is evidence that they are highly dependent on the mycorrhizal symbiosis, and protura have been used as bioindicators for man-made disturbance, such as forest decline (Kholová, 1968; Nosek, 1982; Alberti et al., 1989).

Collembola feed on different soil microbiota, e.g., fungi, bacteria, actinomycetes, and algae (Rusek, 1998). They may graze fungi from the surface of decaying leaves, fecal pellets, and soil particles. Several studies have demonstrated the importance of fungi in

collembola nutrition (Visser and Whittaker, 1977; Visser et al., 1987; Chen et al., 1995; Klironomos and Kendrick, 1995; Klironomos et al., 1999). However, many collembola are generalist feeders and switch their preferences due to availability of food (Parkinson, 1988; Rusek, 1998). Food selection is affected by substrate type or physiological factors like nutritional status of the fungal hyphae or fungal odor (Bengtsson et al., 1991, 1998; Klironomos et al., 1992), and the chemical quality of an individual fungal food resource can influence collembolan productivity (Booth and Anderson, 1979). Through their comminution, grazing, and spore dispersal collembola have significant impact on the soil fungal community (Visser, 1985).

Within mites the oribatids are predominantly fungal-feeding (Mitchell and Parkinson, 1976; Petersen and Luxton, 1982; Kaneko et al., 1995; Maraun et al., 1998). They reject fresh litter not colonized by fungi as food source (Hartenstein, 1962; Pande and Berthet, 1973). Exceptions are the Phthiracaridae, whose juveniles often live inside leaves or needles, and the Suctobelbidae, which feed mainly on liquids (Maraun et al., 2003). However, some oribatids may feed on nematodes or algae (Sengbusch, 1955; Rockett, 1980; Walter 1987, 1988). This indicates that the feeding behavior of oribatid mites is diverse and, as suggested by Schuster (1956) and Luxton (1972), can be divided into (1) microphytophages (feeding on microflora), (2) macrophytophages (feeding on dead plant material), and (3) panphytophages (unspecialized). In addition to these various feeding habits, oribatid mites are known to switch to other food resources if the preferred food is scarce (Maraun et al., 2003).

Macrofauna that feed on fungi include enchytraeids, dipteran larvae, millipedes, centipedes, thysanurans, and earthworms. Enchytraeids are key grazers in northern soils. Didden et al. (1997) found them to be 80% microbivorous (bacteria and fungi) and 20% saprophagous. Several enchytraeid species are fungivorous (Dash and Cragg, 1972; Dash et al., 1980), but there is no information on whether they select particular species of fungi. Enchytraeid grazing affects growth and respiration of fungi (Hedlund and Augustsson, 1995; Jaffee et al., 1997).

Larvae of chironomids, midges, and crane flies have been studied in soils. Anderson (1975) investigated gut contents and found chironomids feeding almost exclusively on fungal material. Spores seemed to be more common than hyphae, and saprotrophic fungi more numerous than pathogenic fungi (Reddersen, 1995). As feeding behavior of dipteran larvae consists of both channeling within and grazing on the surface of the substrate, they may affect fungi by damage to the mycelial thallus, with some fungi more sensitive than others (Visser, 1985). Grazing by midge larvae has the potential to affect fungal species structure, as suggested in a laboratory experiment by Wicklow and Yocum (1982), who observed a reduction in the number of coprophilous fungal species in rabbit dung with increased density of dipteran larvae. Midge larvae were also shown to be a valuable biological control tool for *Rhizoctonia* in muck farming regions of Canada (Anas and Reeleder, 1988).

Earthworms feed on plant residues, which are usually colonized by the soil microflora and microfauna. In particular, protozoa and fungi are regarded as an important food source (Bonkowski and Schaefer, 1997; Winding et al., 1997). Gut contents and casts of earthworms contain fungal hyphae and propagules, which are at least partly digested (Dash et al., 1986; Wolter and Scheu, 1999). The few studies that investigated feeding habits revealed different food preferences depending on earthworm species tested. Saprotrophic soil fungi, fast-growing zygomycetes, and plant pathogens (*Fusarium*) were shown to be suitable hosts (Moody et al., 1995; Bonkowski et al., 2000). There is evidence that earthworms use the composition of the fungal community as a cue to detect fresh organic resources in soil (Bonkowski et al., 2000).

28.4 FUNGAL RESPONSE TO GRAZING

Fungi have developed physical and chemical defenses to grazing. In addition, some fungi change their growth form in response to being grazed. Physical defense mechanisms may limit the invertebrate species that can feed on fungi, as well as grazing effects. Thick, rough, and melanized hyphae may be generalized defenses against grazing, as exemplified by *Cenococcum geophilum*. Sheaths of ectomycorrhizae have outer rinds that may protect them against grazing by smaller soil invertebrates, but not by fly larvae. Wicklow (1979) showed that larvae of the fungus gnat, *Lycoriella mali*, did not feed on sporocarps of *Chaetomium bostrycodes*, apparently because of coarsely roughened perithecial hairs. Since *C. bostrycodes* occurs late in coprophilous succession, when many insects are present, the roughened hairs may be an important defense mechanism. Altered growth form and growth rate of fungi are reported from *Mortierella isabellina*, which changed morphology from appressed, sporulating hyphae to fast-growing aerial hyphae when grazed by collembola (Hedlund et al., 1991).

Secondary chemicals with anti-insectan activity occur in sclerotia and other resting structures of fungi. It is likely that these chemicals evolved as defenses against fungivory, but there is no proof of this. Gloer (1997) found anti-insectan compounds such as ochratoxin A, penicillic acid, and paspalinine family tremorgenic compounds in long-term reproductive structures such as sclerotia and ascostomata, but not in vegetative structures such as conidia and mycelia. He reported anti-insectan compounds from saprophytic genera such as *Aspergillus* and *Penicillium*, but not from phytopathogenic fungi. It is not known whether spores or cleistothecia contain anti-insectan compounds, but it has been proposed that the peptides in mushrooms of *Amanita* species are chemical defenses (Shaw, 1992). For example, Shaw (1988) argued that chemical defenses played a role in determining the feeding preferences of the collembolan *Onychiurus armatus*. He isolated 12 fungi from the same forest site where the *O. armatus* were collected and showed that fungi selected as food were most suitable in the long-term diet of the collembolan. Importantly, Shaw's (1988) study strongly suggested that fungal toxins determined the feeding preferences of *O. armatus*.

Feeding preferences of collembola for functional groups of fungi have been determined by field and microcosm experiments. Curl (1988) and Lartey et al. (1989) found that the plant pathogen *Rhizoctonia solani* was grazed by collembola in preference to the saprophytes *Gliocladium virens* and *Trichoderma harzianum*, thus increasing the survivorship of cotton seedlings in commercial fields. Subsequently, *Gaeumannomyces graminis* var. *tritici* and *Fusarium culmorum* were shown to be grazed by the collembolan *O. armatus*, thus decreasing the severity of fungal disease of wheat seedlings in laboratory experiments (Sabatini and Innocenti, 2001). Klironomos and Kendrick (1996) used microcosms containing sugar maple seedlings to show that three mite and three collembolan species preferred feeding on saprophytic over arbuscular mycorrhizal fungi. The feeding preferences, if substantiated, may reflect the same selective pressures acting on vascular plants where long-lived species that are continually exposed to herbivores are protected by quantitative defensive compounds such as tannin, and short-lived plant species tend to have qualitative chemical defenses based on secondary compounds. Among fungi, species continuously exposed to fungivory, such as *Cenococcum* or *Cladosporium*, have melanic hyphae or roughened pereithecial hairs. Fungi that appear to be using the qualitative strategy, such as *Aspergillus flavus*, have the anti-insectan compounds observed by Gloer (1997), as described above. However, soil invertebrates have evolved strategies to overcome defense mechanisms of fungi and feed selectively on fungi (see Section 28.7).

Compensatory growth, i.e., an increase in growth or respiration in response to grazing compared with an ungrazed control, appears to happen in fungi at moderate grazing levels. A laboratory experiment with episodic grazing of small colonies of *Verticillium bulbillosum* and *Penicillium spinulosum* by the collembolan *O. armatus* supported the concept (Bengtsson et al., 1993). Further, six examples of compensatory growth in response to grazing, particularly by smaller rather than larger invertebrates, were listed by Lussenhop (1992). Compensatory growth may be the basis for some of the stimulation of fungal development by invertebrate grazing. For example, Harris and Boerner (1990) found the greatest plant mass and phosphorus concentration in arbuscular mycorrhizal *Geranium robertianum* grown with intermediate densities, i.e., grazing pressure, of the collembolan *Folsomia candida*. In the field, Birkemoe and Liengen (2000) showed that nitrogen fixation by cyanobacteria was maximized by intermediate densities of grazing collembola in a high arctic salt marsh.

28.5 FUNGIVORY IN THE SOIL FOOD WEB

The basic functional groups and their interactions in the soil food web are not thoroughly described. There is enough understanding of the short-grass prairie food web (Hunt et al., 1987) and the soil food webs in agricultural settings (de Ruiter et al., 1993) that N and C mineralization rates can be predicted within 10% of observed values. In other habitats, particularly in natural soil systems, functional groups and interactions are less well known. For example, a predominance of undiscribed ascomycetes associated with organic nitrogen occurs in winter, under snow in tundra (Schadt et al., 2003). These fungi may be considerable reservoirs of nitrogen. Grazing of these fungi by soil invertebrates under the snowpack is likely to be important (McBrayer and Cromack, 1980). On the other hand, the physical habitat of litter-inhabiting basidiomycetes was found to be reduced by the spread of introduced, surface-casting earthworms throughout North America due to the decrease in the depth of litter on the soil surface by earthworm burrowing (Burtelow et al., 1998).

Soil food webs may have four to eight trophic levels (Hunt et al., 1987), and many of the organisms are omnivorous. Neutel et al. (2002) used soil food webs to show that webs with many omnivores are stabilized by long loops of weak interactions. The number of trophic levels has also led to experiments to determine whether control of numbers at each level is by predators (top down) or resources (bottom up). Experiments suggest the rate of resource supply determines the density of bacteria and fungi, and their grazers, but not the reverse (Scheu and Setälä, 2002). Absence of top-down effects in decomposer systems may be due to the ubiquity of omnivory among the fauna and the occurrence of refuges, like minute pores, that protect some bacteria and fungi from grazers. Moreover, bacteria and fungi are able to compensate for consumed biomass by accelerating their turnover rate. This lack of top-down control is particularly apparent for a bacterial-based food web with protozoa and nematodes as dominant grazers, which in addition built the top predators in this system. In contrast, a fungal-based web may be more prone to trophic cascades as it comprises a species-rich community of predators (Scheu and Setälä, 2002; Wardle, 2002).

Stressing that trophic cascades are of limited importance in soil communities does not imply that the structure of the food web may not affect the belowground system. The ability of animals to regulate processes such as energy flow depends on the sum of their biotic interactions at the same trophic level (e.g., competition) and at different trophic levels (e.g., predation, grazing, competition) (Moore et al., 1988). Hereby their input is

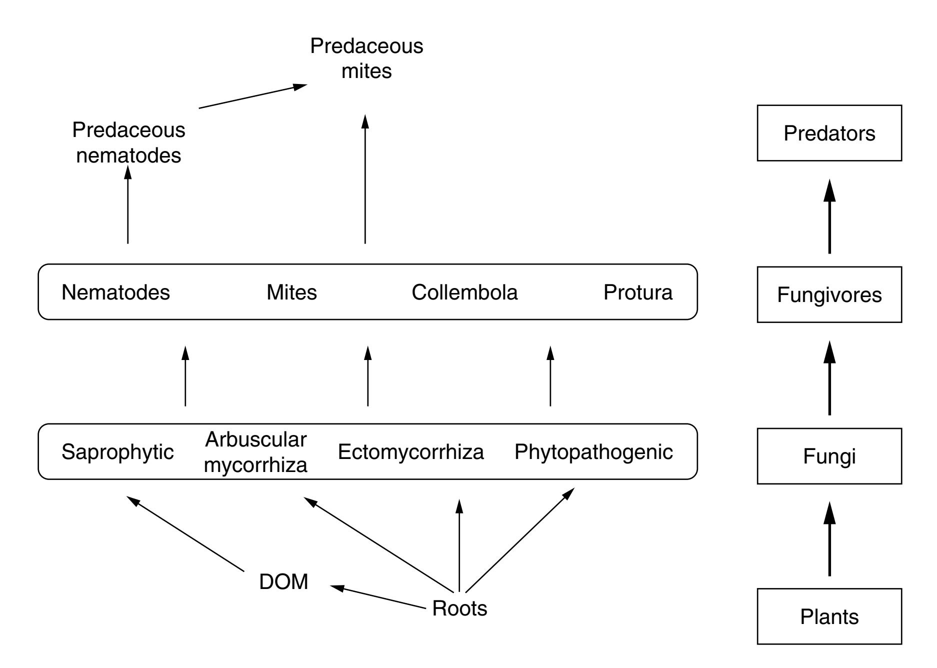


Figure 28.1 The fungal food chain in the soil, with major functional groups shown.

more related to function than taxonomic relationship. The fungal-based food web in Figure 28.1 is organized in terms of faunal groups that have similar feeding habits. These feeding groups serve as a link between species interactions and energy flow through the web. As indicated, it is likely that each group has more than one fungal food source and that the groups share a common predator. This high degree of connectance in the web results in functional redundancy and resilience to perturbation (Ferris et al., 2001).

28.6 FUNCTIONAL GUILD CONCEPT

Fungivores within taxonomic groups may have very different life history strategies. For example, oribatid mites mostly have low grazing rates and long life cycles, whereas collembola tend to have high grazing rates and short developmental times (Moore et al., 1988). Categorizing fungivores into functional guilds whose members have comparable life history, i.e., respond similarly to food web enrichment or environmental perturbation, will give an enhanced framework for food web diagnosis. The functional guild approach was introduced by Ferris et al. (2001) using nematode faunal analysis. Nematodes are the most abundant metazoa in soil and constitute several trophic levels of the food web (Hunt et al., 1987; de Ruiter et al., 1993). The proposed channel index is a valuable tool to assess the relative flow of energy and nutrients through the bacterial and fungal channel and seems to be most useful for tracking successional changes or disturbance within a soil system (Ruess and Ferris, 2004). Two examples are given in Figure 28.2: (a) the responses to disturbance by either acid or alkaline emissions in grassland (data from Valocká and Sabová, 1997) and (b) the changes in decomposition channels along a transect in a meadow–spruce forest ecotone (data from Háněl, 1992). A high channel index stands for a fungal-based energy channel, whereas low values indicate a bacterial-based decomposition. Acid and alkaline perturbations resulted in a change in soil pH, i.e., a decrease in

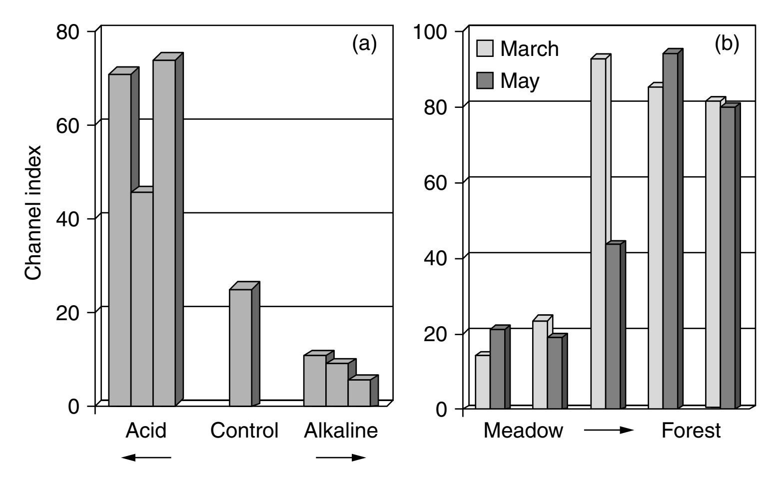


Figure 28.2 Channel index indicating changes in decomposition pathways due to perturbation. (a) Changes due to acid and alkaline emission (Valocká and Sabová, 1997). (b) Changes along a meadow–spruce forest ecotone (Háněl, 1992).

acid and an increase in alkaline plots. The channel index indicates a respective shift favoring fungi in the acidified plots and bacteria in the alkaline plots. Successional changes from a bacterial- to a fungal-based decomposition along the transect from meadow to forest are indicated by an increase of the channel index. This fits to the observed enhanced fungal abundance along the transect. However, the latter study reveals differences in the channel index due to sampling date. Generally, soil and climate seem to affect nematode fauna so strongly that the usefulness of those indices may be restricted to uniform habitats (Ruess, 2003).

Nevertheless, the approach to use functional groups, which are not necessarily taxonomically related but exploit a resource in a similar way, is an interesting feature to investigate decomposition and mineralization processes. Several analyses of food webs demonstrate that different functional groups of the fauna are important in driving these processes in different situations (Hunt et al., 1987; de Ruiter et al., 1993, 1994). It would be worthwhile to extend the functional group concept from nematode communities to other members of the soil fauna. The functional group concept could have widespread application in analyzing nutrient and energy flow through soils and, by this, link belowground and aboveground processes.

28.7 SUCCESSION OF FUNCTIONAL GROUPS DURING DECOMPOSITION

The feeding preferences of dominant fungivores, i.e., functional groups, in relation to the dominant soil fungi in a shared habitat will help to define their importance in the food web. As each succession is dependent on resource and environmental factors (Frankland, 1998), we classified functional groups of fungi due to their enzymatic abilities (Figure 28.3). During decomposition the nature and abundance of substrate change with time from readily decomposable compounds to a proportionally greater recalcitrant fraction. Substrate is initially colonized by pioneer saprophytic fungi (*Cladosporium*, *Alternaria*) or sugar fungi (zygomycetes), which use simple soluble nutrients (Wicklow and Carroll,

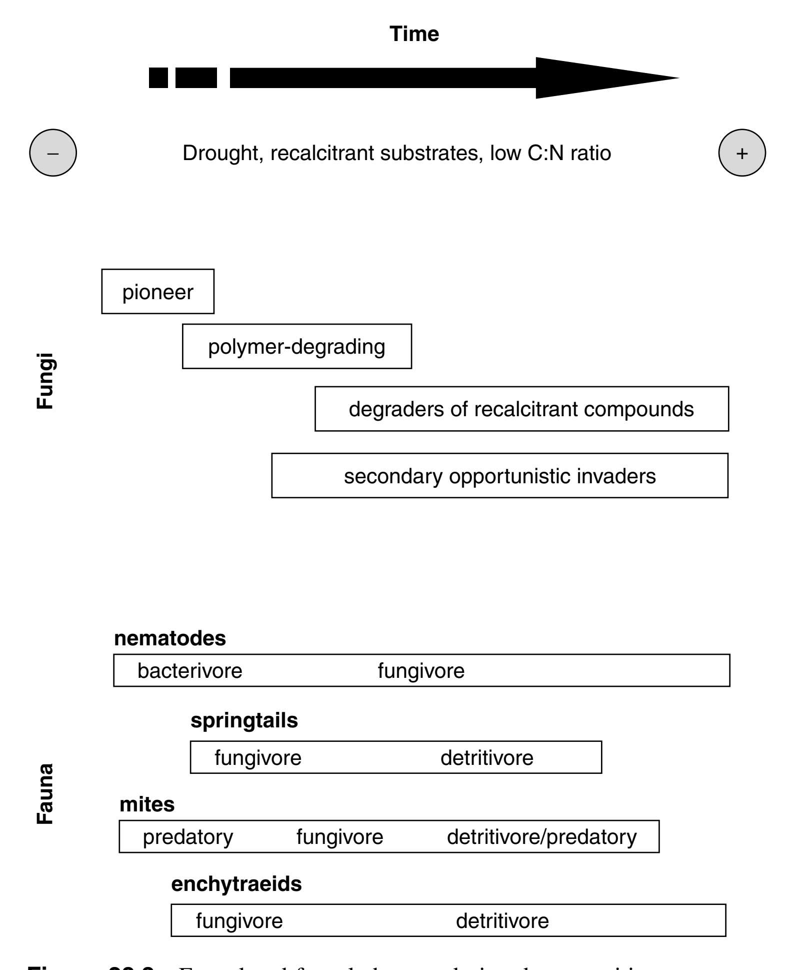


Figure 28.3 Fungal and faunal changes during decomposition processes.

1981; Frankland, 1998). They are followed by the more specialized polymer degraders (*Chaetomium*), which utilize cellulose, hemicelluloses, or chitin. In later successional stages, the fungal flora comprise species able to break down recalcitrant compounds, which are accompanied by secondary opportunistic invaders (mainly basidiomycetes). Generally, the early stages of succession are characterized by a high biochemical and fungal diversity, whereas later phases comprise fewer functional groups (Visser and Parkinson, 1975; Rosenbrock et al., 1995; Dilly and Irmler, 1998). The successional changes within the fungal community are associated with an increase of drought, accumulation of recalcitrant substrates, and a lower C/N ratio.

During organic matter decomposition strong interactions between fungi and the fungivore fauna occur. The succession of functional groups of fungi results in a related succession of functional groups within the fungal feeders. However, there are only scant attempts to relate this succession to any organisms at other trophic levels (Frankland, 1998). Taking into account feeding strategies of soil animals, we adopted the “league” concept of Faber (1991), but excluded his approach to connect fungivores to their habitat. Instead, we assembled the fauna due to their feeding preferences for functional groups of soil fungi. Three functional groups are hypothesized for soil fungivores:

Grazers of colonizers	Feed on pioneer fungi growing on fresh leaf litter Interact with fungal growth by selective grazing
Grazers of saprophytes	Primary food sources are polymer-degrading saprophytes Affect mineralization processes
Grazers of rhizosphere fungi	Feeding preferences for saprophytic fungi growing in the rhizosphere or mycorrhiza fungi Affect rhizosphere colonization and mycorrhizal symbiosis and, by this, plant nutrient uptake

Soil nematode communities show a greater dominance of fungal feeders in mid to late successional stages, whereas the initial phase of decomposition is dominated by bacterial feeders (Figure 28.3) (Freckman, 1988). This shift likely reflects food availability and not association with season or climate, as fungal-to-bacterial ratios in soil decline similarly (Wardle et al., 1995). Additionally, the occurrence of functional groups of fungi and known feeding preferences of fungivore nematodes fit well. Fungivores belong mainly to the Aphelenchidae, where polymer-degrading fungi, such as the cellulolytic *Chaetomium*, are known to be of good food quality, but saprophytic pioneers (e.g., *Epiccocum*, *Alternaria*) or sugar fungi (e.g., *Mucor*) are unsuitable diets (Ruess et al., 2000). Degraders of recalcitrant substrates, such as most basidiomycetes, which are present in the late phase of decomposition, are suitable food, too. Positive correlations were observed between successional maturity of nematode communities and decomposition of cellulose in soil (Neher, 2001).

In contrast to nematodes, the fungivore mesofauna may be of greater relative importance in early to mid-stages of organic matter decomposition, where the rapid succession of different fungal species, each adapted to utilize specific substrates, offers a rich source of nutrients. In laboratory experiments fungivore collembola and mites generally show a trend to select primary saprophytes over secondary saprophytes as food source (Klironomos et al., 1992). Their feeding preference for dematiaceous fungi (dark pigmented hyphae and conidiospores) such as *Cladosporium* or *Alternaria* is well known (Bardgett et al., 1993a; Kaneko et al., 1995; Klironomos and Kendrick, 1995; Maraun, et al., 1998; Hall and Hedlund, 1999). Some studies indicate that primary colonizers such as *Aureobasidium* or zygomycetes (e.g., *Mucor*) are generally less palatable as food source (Kaneko et al., 1995; Klironomos and Ursic, 1998; Klironomos et al., 1999; Maraun et al., 2003), but others found strong preference for *Mucor* (Mills and Sinha, 1971; Chen et al., 1995; Sadaka-Laulan et al., 1998). Generally, hyaline fungi (basidiomycete forms), which occur in later successional stages, are less preferred (Visser and Whittaker, 1977; Klironomos and Ursic, 1998; Maraun et al., 2003).

However, laboratory preference tests may vary based on culture substrate used for fungi due to fungal odor produced (Bengtsson et al., 1991, 1998) and may, therefore, not reflect the field situation. Nevertheless, distinct trophic specificity of the microarthropods toward the quality of soil organic matter and composition of the microflora during different phases of decomposition is known from the field (Ponge, 1991; Rihani et al., 1995; Takeda, 1995; Hasegawa, 1997). Shaw (1985) found the most highly preferred fungi in laboratory tests to have the highest densities in the field habitat, and Dilly and Irmler (1998) observed a significant coincidence between fungal grazers and the occurrence of nonlignolytic fungi in the earlier phases of decomposition. As generalist feeders with the ability to switch diet depending on food availability, the fungivore mesofauna generally change their nutrition from fungi to litter as decomposition proceeds (Hasegawa and Takeda, 1996). Collembolan communities become impoverished with time, likely due to a depletion in food sources associated with an accumulation of recalcitrant soil organic matter (Chauvat et al., 2003).

In contrast, oribatid mites use a wider range of the decomposition phase as a whole community due to their early colonization by nematophagous species (Vreeken-Buijs and Brussard, 1996) and continued changes within from fungivore to detritivore species (Hasegawa, 1997). At later stages the order of species replacement appears to follow successional stages as in plants and shows a continuous increase in oribatid diversity (Scheu and Schulz, 1996).

From the soil macrofauna enchytraeids, earthworms and Diptera larvae are known to be fungal-feeding; however, feeding preferences or behavior are not well understood. There is evidence that enchytraeids preferentially feed on fungi occurring in the early to mid-phase of decomposition. In laboratory experiments enchytraeids preferred dark pigmented fungi, particularly *Cladosporium* (Dash and Cragg, 1972). Field studies from Anderson (1975) showed that during the first months of succession fungal hyphae and spores formed a large proportion of material present in the gut, whereas with progressing succession enchytraeids fed predominantly on leaf litter. However, as the feeding biology of only a few species of the macrofauna has been studied so far, functional group-related differences in feeding activities remain to be investigated.

In conclusion, the fungal community structure during decomposition controls the succession of the fungal-feeding fauna. In response to changes in resource quality, a succession of functional groups of fungi occurs, which is followed by a corresponding succession in functional groups of fungivores. Although regarded as food generalists, the micro- and mesofauna feeding experiments revealed preferences for certain fungal species. The trophic behavior observed in the laboratory, combined with the occurrence of the fauna in the field, fits well to changes among the fungal community during the decomposition of organic matter. This indicates that interactions between fungi and their grazers are strong and the successional pattern of the soil fauna is more likely related to resource availability and feeding attributes than to abiotic soil factors.

28.8 FUNCTION OF TROPHIC INTERACTIONS

By recycling of organic material belowground, trophic interactions in soil form the basis for plant life aboveground. Invertebrate effects on fungi affect plant growth and have ecosystem consequences as well. Soil fungivores input into these processes at three different levels: comminution, dissemination, and grazing. While dissemination is an indirect effect by transport of fungi and their spores, comminution and grazing have a direct influence. Generally, fungal grazing by micro- and mesofauna enhances mineralization and accelerates decomposition rates and energy transport (Trofymow et al., 1983; Moore et al., 1988; Bardgett et al., 1993b; Rihani et al., 1995; Chen and Ferris, 1999; Hedlund and Öhrn, 2000). However, predominantly moderate grazing is expected to increase fungal growth rates, whereas a highly abundant fungivore community may outstrip fungal growth, in particular at low nutrient levels, and cause biostasis (McGonigle, 1995). Grazing may also release nutrients bound in fungal biomass and affect fungal morphology or physiology. In little grazed or pruned hyphae, tips branch and grow faster and grazing can remove accumulated toxic metabolites in senescent hyphae, enhancing further growth (Hanlon, 1981).

Feces are an important component of the soil. After consumption and defecation, resources have a changed chemical quality. The soil mesofauna comminutes fungi directly by feeding. Besides, in their feces material nutrients are mobilized and inhibitory effects of secondary metabolites are removed, which may stimulate fungal growth (Moore et al., 1988). Generally, the consumption of decaying litter is due more to increasing fungal

content (in the course of decomposition) than to a decrease in its mechanical resistance (Sadaka-Laulan, 1998). Grazing effects of the fauna are likely greater in the early decomposition phase, when microbial activity is high, whereas comminution effects are more important in the later phase (Hasegawa and Takeda, 1996).

Soil fungivores affect their food source not only by harvesting biomass, but by selective grazing. Feeding preferences for different parts of the fungal thallus will alter metabolic activity, whereas preferences for certain fungi will change species composition. Low- to intermediate-level grazing would be expected to increase species diversity, as more species could coexist when the biomass of a single species is kept low. Many soil microarthropods are general grazers and switch food sources, which may result in a higher fungal diversity. On the other hand, fungivores with specific preferences may apply high grazing pressure so that a nonpreferred fungus can outcompete a favored species, even if slow growing. The best example is the effect of the collembolan *Onychiurus latus* on two fungi, *Mycena galopus* and *Marasmius androsaceus*, in a Sitka spruce forest. Although the latter was the superior decomposer, it was limited to the upper litter layer by collembolan grazing, whereas *M. galopus* was still frequent in the fermentation horizon (Newell, 1984a, b). By this, collembolan feeding resulted in a reduced decomposition rate in the forest. Klironomos et al. (1992) showed that grazing of collembola caused the replacement of dark pigmented primary saprophytes by light pigmented secondary saprophytes. This indicates that selective grazing can alter the outcome of competition between fungi and may be an important factor in determining the distribution of fungi in the field. Fungal succession observed on decaying litter may result from preferential grazing by fungivores and in turn may affect decomposition rates.

Besides direct effects of grazing on the fungal food source, fungivores indirectly modify plant–fungus interactions. Selective grazing of microarthropods on nonmycorrhizal fungi stimulated arbuscular mycorrhizal colonization (Klironomos and Kendrick, 1995). In particular, grazing of nonmycorrhizal fungi in the rhizosphere may protect plants against pathogens and parasites and may help to establish mycorrhiza. Generally, via nutrient mobilization, the fauna indirectly affect both the performance of mycorrhizal fungi and the stability of the plant–fungus symbiosis. However, when nutrients are scarce, fungivores may overconsume and render the symbiosis ineffective, whereas when nutrients are abundant and mycorrhizal fungi become parasitic, grazing may be beneficial for plants (Scheu and Setälä, 2002).

28.9 CONCLUSIONS AND PROSPECTS FOR THE FUTURE

Soil fungi serve various types of soil fauna as a food resource. The direct trophic interactions between fungi and fungivores can change fungal morphology and physiology and alter community structure. They can also induce fundamental changes in the performance of the plant–fungus association. By this, fungal grazers affect mineralization, decomposition rates, and energy transport in soils. Because taxonomically related species do not necessarily use similar fungal food resources, the partitioning into functional groups whose members have similar feeding habits and life history strategies may help to further clarify the specific role of the fauna. However, soil fungivores (and even more detritivores) are very flexible in their diet and may act as microbivores, herbivores, detritivores, or even predators (Scheu, 2002).

There is a need for more detailed information on the diet of soil animals, and some new methodical developments may offer considerable profit in the near future. Recently, the natural abundance of stable isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$) was used to investigate the food

web structure of soil animals (Ponsard and Arditi, 2000; Scheu and Falca, 2000). ^{13}C allows the tracing of food material actually used for biomass production, and ^{15}N gives a measure of the trophic position of an animal. The methodology has been successfully used to define feeding guilds in earthworms (Scheu, 2002). In a fungal-based food web with its several trophic levels, determination of ^{15}N will be a powerful tool to identify functional groups that inhabit similar trophic levels. However, trophic interactions may be unspecific and variable in time, and this may diminish the strength of the isotopic signal. Another approach to assign feeding strategies is to measure biochemical components specific to the food source. The first studies on fungal-feeding nematodes showed evidence for fatty acids to be useful biomarkers in soil food chains (Chen et al., 2001; Ruess et al., 2002).

As already stressed, the trophic interactions between fungivores and their resources are multiple and difficult to evaluate. An alternative to complete structural analysis is provided by the assessment of the presence and abundance of functional groups. Nematode faunal analysis provides a useful tool to investigate the structure of food webs, in particular decomposition channels (Ferris et al., 2001). Descriptive approaches, such as the channel index, derived through faunal analysis in combination with experimental studies using new methods, such as stable isotope and fatty acid analysis, will lead to new insight into the relationships between fungi and their grazers.

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29

Sporocarp Mycophagy: Nutritional, Behavioral, Evolutionary, and Physiological Aspects

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29.1 INTRODUCTION

The phenomenon of mycophagy — in general the eating of fungi, but for purposes here restricted to the eating of fungal sporocarps— has been observed for centuries. It was embedded in mycological nomenclature by Ness von Esenbeck (1820), who coined the generic name *Elaphomyces*, from the Greek for "deer fungus." This widely distributed hypogeous ascomycete was well known to be scraped out of the soil and eaten by deer and came to be called the *Hirschtrüffel* ("stag truffle"). Mycophagy was subsequently noted by various authors such as Parks (1919), but serious study of the feeding habit began with Buller (1922a, b), who reported detailed observations of the fungal foraging behavior of red squirrels (*Sciurus vulgaris*) and slugs.

Since this early pioneering research, sporocarp mycophagy has been shown to be widespread among a diversity of invertebrates and vertebrates, as highlighted in detailed reviews such as Fogel and Trappe (1978), Wilding et al. (1988), Claridge and May (1994), and Claridge et al. (1996). The purpose of the current chapter, which builds on these earlier reviews, is threefold: (1) to provide a synopsis of the degree to which different animals, particularly mammals, consume fungal sporocarps; (2) to review the nutritional characteristics of fungi compared with other foodstuffs; and (3) to describe how differ-

ences in the digestive anatomy and physiology of mammal species might help explain the degree to which such foods are utilized in nature. Stemming from these broad subject areas, recommendations for future research are indicated. Literature cited here is mainly that from studies undertaken in the Pacific Northwestern U.S. and Australia, where studies of various aspects of mycophagy by mammals have been more extensive than in other parts of the world.

29.2 CATEGORIES OF MYCOPHAGY

Mycophagists, including both invertebrates and vertebrates, can be categorized by their relative dependence on fungal sporocarps for nourishment:

1. Obligate: Entirely or almost entirely dependent on sporocarps.
2. Preferential: Prefer sporocarps over other dietaries, but regularly or seasonally eat other types of food as well.
3. Casual or opportunistic browsers: Occasionally feed on sporocarps as available and attractive.
4. Accidental: Ingest sporocarps or spores accidentally in the course of eating other dietaries.

29.2.1 Obligate Mycophagists

This small minority of vertebrates and invertebrates represents either coevolution with the fungi eaten or an adaptation so strong as to be a virtual coevolution. In the case of mammals, such mycophagists require habitats in which fungal sporocarps are available throughout the year or, if weather precludes sporocarp production for short periods, that offer alternative dietaries as a carryover food source. Obligate mycophagist mammals focus on sporocarps of hypogeous, ectomycorrhizal fungi, so they require ectomycorrhizal host plants in their habitat. In the northern hemisphere, obligate mycophagy is most striking in the Californian red-backed vole (*Clethrionomys californicus* var. *californicus*). Its fragile teeth are not adapted to abrasive or hard foods (Maser, 1998). Moreover, in contrast to most other rodents, its rooted teeth do not continue growing throughout life. Fungal sporocarps are generally among the less abrasive foods, and var. *californicus* lives in one of the infrequent habitats where fungi may fruit throughout the year — the coastal fog belt forests of Oregon and northern California (Maser, 1998). When hypogeous sporocarps are not available, the vole eats fruticose lichens that storms have blown to the ground from tree crowns (Maser et al., 1978).

In the southern hemisphere, two marsupial rat-kangaroo species, the long-footed potoroo (*Potorous longipes*) and Gilbert's potoroo (*Potorous gilbertii*) best exemplify obligate mycophagist mammals. The former species, described in 1980, has an extremely patchy distribution within damp and wet sclerophyll (eucalypt) forest habitats in far southeastern mainland Australia — characterized by relatively high annual rainfall and moderate winter temperatures (Claridge, 2002b). The most comprehensive study of the diet of the species highlighted that on average, fungal sporocarps compose over 90% of dietary items in feces (Green et al., 1999). Minor dietary items included plant parts and some invertebrates, although these food groups seldom make up more than 5% by volume of feces. Similarly high proportions of fungi have been found in the feces of the recently rediscovered (Sinclair et al., 1996) Gilbert's potoroo, an inhabitant of heathland and scrubland in far southwest Western Australia (Bougher et al., 1998).

A large number of insects, especially beetles and flies, appear to be obligate mycophagists. The life cycles and feeding strategies of insect mycophagists are astoundingly diverse and complex and exceed the scope of this chapter. The topic is reviewed in Wilding et al. (1988).

29.2.2 Preferential Mycophagists

A wide array of mammals and possibly some crustaceans prefer fungal sporocarps when available. This represents adaptive behavior by the animals in response to visual or olfactory attractants produced by the fungi. In the northern hemisphere, many squirrels (Sciuridae) focus on fungi as a dietary during the fungal fruiting season (for summary, see Luoma et al., 2003). The northern flying squirrel (*Glaucomys sabrinus*) strongly prefers hypogeous fungi (Maser et al., 1985, 1986) but also feeds on a variety of other dietaries (Thysell et al., 1997). The Australian marsupial families Potoroidae (including several species of bettongs and potoroos) and Peramelidae (including bandicoots and bilbies) contain many preferential mycophagists (for further information, see summaries by Claridge, 2002a). Relevant examples include the northern bettong (*Bettongia tropica*) from dry sclerophyll woodland habitats in tropical Queensland (Johnson and McIlwee, 1997) and the southern brown bandicoot (*Isoodon obesulus*), an inhabitant of dry sclerophyll forests and heathlands of southeastern mainland Australia and Tasmania (Claridge and May, 1994).

29.2.3 Casual or Opportunistic Mycophagists

Most nondomesticated mammals and birds plus slugs and snails fit this category. Most rodents that have been studied in both the northern and southern hemispheres are recorded as having ingested fungi on occasion, sometimes with considerable focus (Fogel and Trappe, 1978; Maser et al., 1978; Blashke and Bäumler, 1989; Perez Calvo et al., 1989; Janos et al., 1995; Claridge, 2002a; Luoma et al., 2003). At least one shrew (*Sorex townsendii*) includes hypogeous fungi in its diet (Maser et al., 1978). The larger southern hemisphere marsupials, such as possums and wallabies, often eat fungi (Claridge, 2002a; Claridge et al., 2001). Presumptive carnivores such as fishers (*Martes pennanti*) and foxes may eat considerable amounts of hypogeous fungi, as do the omnivorous skunks (*Spilogale* spp.) and bears (*Ursus* spp.) (Zielinski et al., 1999; Trappe, unpublished data). Elk (wapiti), moose, caribou, reindeer, and especially deer are occasional to seasonally focused mycophagists (Ahti and Hepburn, 1967; Miller and Halls, 1969; Le Resch and Davis, 1973; Cushwa and Coady, 1976; Cederlund et al., 1980; Trappe, unpublished data). As Le Resch and Davis (1973) reported, "Mushooms, especially *Boletus* spp., were abundant during the summer of 1970, and apparently were eaten whenever encountered by moose." Various primates are recorded as mycophagists (Altman and Altman, 1970; Fossey, 1983; Bermejo et al., 1994; Porter, 2001), perhaps including the yeti, or abominable snowman (Zang and Doi, 1995). Some birds seek both epigeous and hypogeous fungi as food, probably a more common behavior than has been recognized so far (Miller and Halls, 1969; Simpson, 1998, 2000; Medway, 2000).

29.2.4 Accidental Mycophagists

Animals that ingest soil or digestive tracts or feces of mycophagist mammals in the course of feeding may occasionally also ingest spores or sporocarps of hypogeous fungi. Predatory vertebrates, earthworms, and dung beetles exemplify this group (Matthews, 1972; McIlveen and Cole, 1976; Reddell and Spain, 1991). Because fungi are incidental to the behavior and nutrition of accidental mycophagists, they will not be considered further in this chapter.

29.3 NUTRIENT CONTENT OF SPOROCARPS

Fresh fungal sporocarps are sometimes regarded as nutritionally impoverished because most are 80 to 90% water. Even so, they can have high contents of certain nutrients, and dried ones can be particularly high in many nutrients. For the most part, animals would eat fresh sporocarps and probably obtain most of their water as dietary and metabolic water (Wallis et al., 1997). Caching animals such as squirrels and pack rats (*Neotoma* spp.) often employ the use of dry sporocarps by hanging them on tree branches or laying them in the sun (Buller, 1922a; Hardy, 1949; Smith, 1968; Hall, 1981). Once dried, the animal caches them in a dry, sheltered spot such as a tree hollow for later use.

Evaluating the nutritional value of sporocarps of individual species is difficult because their nutrient contents can vary substantially between times and places picked, soil properties, weather, developmental stage, morphological part (i.e., stems vs. caps of mushrooms or peridium vs. gleba of hypogeous fungi), and analytical methods used (Watkinson, 1964; Sawada, 1965; Byrne et al., 1976; Ohtonen, 1978; Quinche, 1983a, 1987; Bencivenga and Granetti, 1989; Agaoglu et al., 1992; Giaccio et al., 1992; Wallis et al., 1997; Manzi et al., 2001). Comparing the nutritional value of fungi with other food resources used by mycophagists is even more difficult, because plants can vary within the same habitat and analytical factors as the fungi. Consequently, definitive comparisons should be from fungus and plant materials collected at the same places and times and analyzed in the same way. This has been done only rarely. Nonetheless, some generalities are possible. The comparisons outlined below are based mostly on Le Resch and Davis (1973) and Hanley and McKendrick (1983) for data on plants because those two sources provide analytical data for a wide variety, from moss and algae to trees. The literature offers few clues as to the relative concentrations of any nutrient of mycorrhizal fungi vs. decomposers or epigeous vs. hypogeous species, again because of high variation within, as well as between, species of these different groups.

29.3.1 Macroelements

Concentrations of N, P, K, Ca, Mg, Na, and S vary markedly between fungal species, habitats, and analytical methods. Huge differences may occur between species in total N (Vogt and Edmonds, 1980), although Cromack et al. (1975) and Claridge et al. (1999) showed high N concentrations in several species. Much of the nitrogen in sporocarps is unavailable to mycophagists, because it is tied up in poorly digestible compounds such as chitin (Cork and Kenagy, 1989; Claridge et al., 1999). The amino acid and protein contents, as presented in Section 29.3.5, offer the nutritionally more meaningful data on N.

Fungi, especially mycorrhizal species, are particularly competent in P uptake compared with plants. It is not surprising, therefore, that P concentrations tend to be high in fungi (Vogt and Edmonds, 1980; Sanmee et al., 2003). The other macronutrients vary so much between species and localities that few generalities are possible. Potassium is often high and Ca and Na low in sporocarps (Cromack et al., 1975; Grönwall and Pehrson, 1984; Sanmee et al., 2003; Vetter, 2003), especially in comparison with eggs, milk, or vegetables (Kreula et al., 1976). These three elements, however, can vary markedly among and within species (Manzi et al., 1999). Magnesium and S show no consistent patterns of accumulation (Vogt and Edmonds, 1980; Bencivenga and Granetti, 1989; Coli et al., 1989, Sanmee et al., 2003).

29.3.2 Microelements

Selenium has received particular attention as an essential trace element that can be deficient in some soils but toxic when ingested in excess. Watkinson (1964) was the first to discover

Se accumulation in a fungus — *Amanita muscaria* in New Zealand. On a dry weight basis, Se ranged from 16.8 to 17.8 ppm, up to 600 times the concentration in foliage of associated herbs and trees. Even higher concentrations have been reported for some other fungi, e.g., in Europe up to 19.4 ppm for *Boletus edulis* (Quinche, 1983a) and 22.1 ppm for *Agaricus campestris* (Quinche, 1983b). These high concentrations are exceptional, however, even within the species mentioned above (Stivje and Cardinale, 1974; Byrne et al., 1976; Stivje, 1977; Quinche, 1983a, 1983b, 1987; Sanmee et al., 2003). Nonetheless, the concentrations in fungi in general are high to extremely high compared with the foliage concentrations reported by Watkinson (1964) or soil on which the fungi grew (Quinche, 1983b). Soils of the Olympic Peninsula of Washington State are deficient in Se, and livestock feed needs to be supplemented with Se to prevent the Se deficiency white muscle disease. Deer and elk (wapiti) in the area are not known to suffer from that disease; they commonly eat mushrooms, perhaps using them as a salt lick that prevents Se deficiency (E. Starkey, personal communication).

The fungal contents of other micronutrients also vary substantially within and among species, but Kreula et al. (1976) found the fungi to be particularly high in Mn, Cu, and Zn, compared with milk, eggs, potatoes, and other vegetables. Sporocarps of *Tuber melanosporum* from five different localities contained much higher contents of B, Cu, Fe, Mg, Sr, and Zn than surrounding soil. Soil adjacent to the sporocarps was generally lower in these elements than in soil at a distance, suggesting that fungal uptake depleted the soil (Bencivegna and Granetti, 1989). Sporocarp content of Al, Cr, Mo, Ni, Pb, and Sn, in contrast, was negligible compared with the soil. Byrne et al. (1976), in contrast, found high variability in microelement ratios of sporocarp to soil in analyses of 27 species of fungi, so their data permitted no generalities. Giaccio et al. (1992) reported for seven *Tuber* spp. that in general the sporocarps accumulated Cu and Cd; Zn was accumulated only in a certain range of concentrations; Cr and Ni were sometimes accumulated, sometimes not; and Mn and Pb were never accumulated.

29.3.3 Carbohydrates

Total nitrogen-free carbohydrates range from about 28 to 85% of fleshy fungi on a dry weight basis (Sawada, 1965; Al-Delaimy and Ali, 1970; Bokhary and Parvez, 1993; Murcia et al., 2003; Sanmee et al., 2003). However, much of the carbohydrates in fungi is in poorly digestible compounds such as cellulose, so sugar and sugar alcohol contents are more meaningful in terms of available energy. Sanmee et al. (2003) reported 10 sugars and 8 sugar alcohols in fleshy fungi, although individual species varied, having from 5 to 9 of the sugars and from 4 to 7 of the sugar alcohols. As true of other nutrients, values for individual sugars varied strongly between fungal species. For example, trehalose ranged from 0.5 mg/g of dry weight in *Astraeus hygrometricus* to 12.4 mg/g in *Lactarius glaucescens*. Sawada (1965) analyzed trehalose content of 38 species of fungi, including woody conks on trees. He failed to detect any trehalose in some species, but most ranged similarly to those of Sanmee et al. (2003). Glycerol made up 16.8% of the dry weight of *Terfezia claveryi* (Bokhary and Parvez, 1993).

29.3.4 Fats and Fatty Acids

Total fat of all but a few fungal species tested for it ranges from 0.5 to 20% of sporocarp dry weight, depending on species (Sawada, 1965; Al-Delaimy and Ali, 1970; Kreula et al., 1976; Sawaya et al., 1985; Coli et al., 1989; Agaoglu et al., 1992; Murcia et al., 2003; Sanmee et al., 2003). Wallis et al. (1997) discovered that the peridium of unidentified hypogeous fungi had negligible fat content, whereas the gleba contained about 22%. These figures are low compared with milk and eggs, but much higher than potatoes (Kreula et

al., 1976). One conk-forming wood decomposer, *Laricifomes* (*Fomitopsis*) *officinalis*, registered a startling 55% fat content (Sawada, 1965), higher than that of eggs (Kreula et al., 1976). Spores and hyphae of Glomeromycetes and Endogonales are full of lipid globules (Gerdemann and Trappe, 1974), but the percentage content by dry weight is unknown. Kreula et al. (1976) and Murcia et al. (2003) identified five to nine different fatty acids in several fungi, with oleic and linoleic acids predominating. Eleven to 13 fatty acids were found in truffles by Coli et al. (1989) and Bokhary and Parvez (1993); oleic and linoleic acids again predominated, along with palmitic and stearic acids.

29.3.5 Proteins and Amino Acids

Crude protein contents of sporocarps, like fats, depend on species and range from 6 to 42% of dry weight (Sawada, 1965; Al-Delaimy and Ali, 1970; Al-Delaimy, 1977; Grönwall and Pehrson, 1984; Coli et al., 1989; Agaoglu et al., 1992; Manzi et al., 1999; Sanmee et al., 2003). Proteins mostly must be broken down into amino acids to be digested by mycophagists, but fungi have considerable amino acid content as well. Seventeen to 29 different amino acids, including most to all of the essential ones, have been detected in *Tuber*, *Terfezia*, and several mushroom species (Al-Delaimy, 1977; Patel, 1980; Sawaya et al., 1985; Coli et al., 1989; Bokhary and Parvez, 1993; Manzi et al., 1999). Amino acid content of the gleba of unidentified hypogeous fungi was reasonably balanced and over 9% of dry weight; that in the peridium was negligible (Wallis et al., 1997).

29.3.6 Vitamins

Few studies on vitamin content of fungal sporocarps have been reported. Nonetheless, various species are rich in vitamins A, B complex, C, D, and K (Sawaya et al., 1985; Agaoglu et al., 1992; Mattila et al., 2001). As the only nonanimal source of vitamin D (Mattila et al., 2000), epigeous fungi are likely an important source for nocturnal and fossorial animals. Hypogeous fungi have not been investigated for vitamin D content, but because the metabolic route from ergosterol to vitamin D requires sunlight (Mattila et al., 2002), they may not be good sources.

29.4 NUTRIENT AVAILABILITY

The chemical content of sporocarps is only part of the story regarding the nutritional virtues or otherwise of a fungal diet. The other part is the degree to which key nutrients and other chemical components of ingested fungi can be assimilated by the mycophagist. For some components of fungal sporocarps, it is clear that little nutrition can be derived. For example, spores and hyphae of the Endogonales and Glomeromycetes are full of lipid globules. In scats of rodents that have consumed them, the spores are nearly all intact (Trappe, unpublished data), so only the lipids in the hyphae are potentially available to the mycophagist.

The degree to which nutritional benefit is obtained from other components of fungal sporocarps other than spores relates strongly to differences in digestive anatomy and physiology between mammal species. The earliest experiments in which known amounts of fungi were fed to mammals in captivity suggested a poor overall nutritional quality compared with other foodstuffs. Captive golden-mantled ground squirrels (*Spermophilus saturatus*) were fed the fruit bodies of *Elaphomyces granulatus*, the common stag truffle, and the digestibility of the fungi was compared with that of the leaves of a variety of plant species eaten naturally by the squirrels, as well as cones, pine nuts, leguminous foliage, and grass (Cork and Kenagy, 1989). A high-quality food, rodent laboratory chow, was

used as a reference diet. Squirrels were offered preweighed amounts of the different foods. Squirrels maintained or gained body mass on only two of the food types: pine nuts and rodent chow. Despite consuming a high daily intake of *Elaphomyces*, squirrels lost weight. The digestibility of nitrogen and energy from *Elaphomyces* was lower than that recorded for nearly all the other diets. Furthermore, although chemical analyses revealed that the nitrogen content of fruit bodies was relatively high, 80% of it was bound in totally indigestible spores that the squirrels rarely ate. Of the remaining 20%, only half was present as protein nitrogen. Sources of energy were tied up in complex, relatively indigestible cell wall tissue.

The overall digestibility of *E. granulatus* fruit bodies fell just below the critical threshold for the squirrels to maintain energy balance. For these squirrels, which have a relatively simple digestive tract, *E. granulatus* was seen as a marginal but important diet when no alternative was available (Cork and Kenagy, 1989). Moreover, the fruit bodies were readily detectable and required minimal processing time prior to consumption, unlike some foods, such as seeds from cones, maximizing the yield of energy and nutrients in relation to foraging effort. If the squirrels could not maintain normal energy balances by eating solely hypogeous fungi, then the minor incorporation of rarer, but higher-quality foods may be all that would be required for them to persist.

More recent feeding trials by Claridge et al. (1999), with two other North American rodent species, the northern flying squirrel (a preferential mycophagist) and the Californian red-backed vole (an obligate mycophagist), reaffirm that simple-stomached animals have difficulty digesting fungal foods. When fed a diet of a single species of fungus, *Rhizopogon vinicolor*, captive animals of either species did not maintain body weight. Interestingly, however, the voles digested the various components of the fungal sporocarps at least as well as the much larger flying squirrels. This ability may be attributed to a modified digestive strategy, whereby fine food particles are selectively retained in the hindgut for a longer duration of time than larger particles. This enables the vole to more efficiently digest fungal foodstuffs than would ordinarily be possible, a capability decidedly advantageous to an obligate mycophagist.

In Australia, some marsupial species are apparently better able to digest fungal sporocarps than are North American rodents. Most rat-kangaroos (see exception below), for example, have special adaptations to the gut, including a large sacciform forestomach (hereafter referred to as an enlarged foregut). In contrast, the hindgut is reduced to a well-developed, though simple, caecum and proximal colon. The enlarged foregut serves as an incubator for anaerobic microbes, which ferment food and convert fungal nitrogen to a form more available for the host animal. This process is called pregastric fermentation. Nutritional physiologists have suggested that the foregut of rat-kangaroos might serve as a food storage area, an advantage to an animal subject to predation and needing to minimize feeding time (Hume and Carlisle, 1985).

The first real evidence that fungal fruit bodies were nutritious for rat-kangaroos was provided in a controlled feeding trial conducted by Claridge and Cork (1994). Captive long-nosed potoroos were fed known amounts of fruit bodies of two species of hypogeous fungi, *Mesophellia glauca* and *Rhizopogon luteolus*. Chemical analyses revealed that although the nitrogen concentration was high in both fungi, much of it was in nonprotein form or associated with cell walls and was thus presumably of low nutritional value or protected from digestive enzymes. The concentration of cell wall constituents (fiber) was high in both fungi, suggesting low availability of digestible energy. Nonetheless, potoroos lost little weight and digested much of the dry matter, nitrogen, and energy in the pure fungal diets. Consequently, animals maintained positive nitrogen balances and high intakes of digestible and metabolizable energy. Most other mycophagous mammals in Australia

lack an enlarged foregut, and most food is digested in the hindgut. The lack of this digestive system may help explain why hindgut fermenters such as rats and bandicoots seldom rely wholly upon fungi but commonly eat other foods such as seeds and invertebrates.

Evidence of the high nutritional value of fungi for rat-kangaroos also comes from several field-based studies. When production of hypogeous fruit bodies was highest, Tasmanian bettongs (*Bettongia gaimardi*) were almost entirely mycophagous, whereas at times of low fruit body production they mainly consumed other foods, such as leaves and fruits (Johnson, 1994a). Body condition of adults tended to increase with increasing amounts of fungus in the diet. When production of fruit bodies increased, energy turnover in adult females and growth rates of pouch young increased concomitantly, suggesting that the fungi provided animals with a surplus of energy, perhaps used in lactation. In another field-based study, but on the northern bettong (*Bettongia tropica*), McIlwee and Johnson (1997) used stable isotope analysis to determine that nearly all nitrogen assimilated into body tissue by animals was from fungi. In contrast, the sympatric northern brown bandicoot (*Isoodon macrourus*) derived much of its nitrogen from invertebrates and practically none from fungi. This finding was mirrored by patterns in the diet of the same animals. Notably, the only rat-kangaroo without an enlarged foregut, the musky rat-kangaroo (*Hypsiprymnodon moschatus*), does not consume fungi to any great degree (Dennis, 2002). Having a relatively simple digestive tract, this rainforest dweller of tropical northern Queensland instead specializes on more readily digestible dietary items, such as fruits and invertebrates.

29.4.1 Diversity in Fungal Diet

Is a diverse fungal diet nutritionally important? It has been suggested that marked variation in nearly all nutrients between fungal species may account for the tendency of obligate and preferential mycophagists to routinely consume a wide diversity of species in order to achieve a balanced diet (Maser et al., 1978; Claridge, 2002a). Certainly, in the field, North American mammals, such as the Californian red-backed vole and northern flying squirrel, and Australian marsupials, such as the long-footed potoroo and northern bettong, routinely consume many species of fungi at any one point in time in the year (Maser et al., 1978, 1985, 1986; Johnson, 1994b; Vernes et al., 2001). Experimental trials on captive animals, along the lines of those described above only using several fungal species instead of a single species, would prove instructive in demonstrating how important diversity in the fungal diet is for nutritional welfare.

Do mycophagous mammals prefer some fungi over others? Preferences in choice of fungi by the Tasmanian bettong were tested by measuring the abundance of spores in feces of live-captured animals against the abundance of particular fungi in protected soil plots. The bettongs consumed all the fungal species found fruiting in the plots and many others that were not collected. Species found in feces were strongly correlated with their abundance in the exclosures, suggesting that bettongs generally consumed fungi in relation to their relative abundance. However, there were two exceptions. The genus *Elaphomyces* was common in the field but comparatively rare in the feces, while the reverse was true for *Zelleromyces*. This was attributed to a real preference choice. However, *Elaphomyces* spp. have a powdery spore mass likely to be discarded by small mycophagists (Trappe and Maser, 1977), which focus on eating the thick peridium. A low content of *Elaphomyces* spores in the feces thus does not necessarily reflect low consumption of the sporocarps. In any event, how such choices might relate to the relative nutritional value of the various ingested genera of fungi is unknown. *Mesophellia* and *Castoreum* were dominant in both the field and feces. These produce large aromatic fruit bodies that are readily locatable. Harvesting pressure on these fungi was particularly high during times of peak production, with the bettongs reducing standing crop by up to 70%. Harvesting pressure on other

fungal taxa was not as great, even at times of peak production, presumably because they were less attractive. As a dispersal vector for a wide variety of fungi, bettongs probably had more influence in distributing *Mesophellia* and *Castoreum* than the other taxa, helping those two genera maintain their status as the most abundant on site. This suggests that by their foraging behavior, rat-kangaroos such as the Tasmanian bettong may help shape the structure and function of fungal communities (Johnson, 1994a).

29.4.2 Interspecific Interactions

In addition to digestive strategy, the degree to which a particular mammal species consumes fungal sporocarps may be influenced by the presence of other mycophagous species in the same habitat. For example, in the eastern woodlands of North America, Orrock et al. (2003) found that the amount of fungus in the stomach contents of the woodland jumping mouse (*Napaeozapus insignis*) varied in relation to the presence and relative abundance of the sympatric deer mouse (*Peromyscus maniculatus*). At sites where the latter species was absent, fungus was a major item in the diet of the jumping mouse. In contrast, at sites where the deer mouse was numerically dominant, the fungal dietary content of the jumping mouse was negligible.

Similarly, in southeastern mainland Australia, Tory et al. (1997) compared the diets of sympatric long-nosed potoroos (*Potorous tridactylus*) and bush rats (*Rattus fuscipes*). While the taxonomic breadth of fungal species consumed by both mammal species was almost the same, the potoroos generally fed on a much larger quantity of fungus than did the bush rats. The exception to this pattern was during the late autumn and winter, when fungal food resources were superabundant. Other research in Australia has demonstrated that sympatric species of marsupial bandicoots consume different fungal species obtained from different microhabitats within the same general forest area, thereby avoiding interspecific competition for fungal food resources (Claridge and May, 1994).

29.5 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Available literature indicates that sporocarp mycophagy is particularly widespread among a range of different mammal species, as well as invertebrates, but may also be prevalent in other vertebrate groups such as birds. The degree to which fungi are incorporated into diets varies among different animals. In a few cases, species have apparently coevolved with their fungal food sources and become obligate or near-obligate mycophagists. The majority of mycophagists, however, use fungi as a preferred food source while also consuming other food types, or opportunistically when the fungal resource is superabundant in the environment. Also, a wide array of animals only eat fungi casually or ingest them accidentally when foraging for other food sources.

Fungal sporocarps differ widely in their chemical composition within and between species, across time and space. This makes it difficult to generalize about the nutritional value of fungal foodstuffs. In general, fungal sporocarps can have high nitrogen concentrations, but much of that nitrogen may be available in poorly digestible forms such as nonprotein nitrogen and chitin. The same can be said for carbohydrates, which are mainly in the form of poorly digestible items such as chitin. On the other hand, fungal sporocarps can have high concentrations of key elements such as phosphorus; micronutrients such as magnesium, copper, zinc, and selenium (the latter may help ease salt deficiency in deer); amino acids; and vitamins. However, the relative availability of these to different mycophagists is largely unknown.

In cases of obligate mycophagy, the species in question have specialized features to enable better utilization of fungi, e.g., modifications of the digestive system to better allow for chemical processing and uptake of nitrogen and carbohydrate sources.

Key areas for future research on sporocarp mycophagy include (1) better describing the feeding habits among poorly studied animal species; (2) examining patterns of foraging and fungal resource partitioning and use in space and time among diverse communities of mycophagous mammals; (3) better defining the nutritional value of fungi as a food resource, particularly the virtues of a diverse array of fungal species vs. single fungal species in the diet; and (4) exploring how the fungal components of habitats interact with other habitat variables in response to disturbances such as fire or logging, and how these responses collectively affect the quality of the habitat for specific animal species.

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Hypogeous Fungi: Evolution of Reproductive and Dispersal Strategies through Interactions with Animals and Mycorrhizal Plants

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30.1 INTRODUCTION

Mycophagy of fungal sporocarps by a great diversity of animals is documented for all continents except Antarctica and entails a range of behaviors and nutritional modes (Fogel and Trappe, 1978; Maser et al., 1978; Emmons, 1982; Perez Calvo et al., 1990; Janos and Sahley, 1995; Claridge et al., 1996; Luoma et al., 2003). With few exceptions, it involves mycorrhizal associations originated with the earliest vascular plants more than 400 million years ago. The fossils of these mycorrhizae show morphologies consistent with present-day arbuscular mycorrhizae and were probably formed with glomalean fungi that formed individual spores or spore clusters in the soil. The spores would have been dispersed by soil movement and perhaps mycophagy by primitive invertebrates. Host genera that today form ectomycorrhizae first appeared about 150 to 200 million years ago, as did mammals. The fossil record so far is silent on the earliest appearance of ectomycorrhizae and ectomycorrhizal fungi, but it was likely close to the same time. Distributions of related ectomycorrhizal host genera and associated fungal genera in both the northern and southern hemispheres suggest common ancestries in the supercontinent Pangaea before it began to

separate into the southern Gondwana and northern Laurasia some 180 million years ago. Hypogeous genera do not likely disperse across even limited ocean barriers, yet the genus *Elaphomyces* includes closely related species with identical, complex peridial structures in both the northern and southern hemispheres, suggesting origins in Pangaea prior to its breakup (Trappe et al., 2001).

Molecular phylogenetic and morphological data evidence that many if not most sequestrate fungi, i.e., hypogeous or epigeous fungi that lack mechanisms and morphologies for discharging spores to the air, have evolved from epigeous, nonsequestrate species. Some hypogeous genera, however, appear to have originated early in the evolution of ectomycorrhizal fungi. The hypogeous genus *Rhizopogon*, for example, likely shares an ancestor in common with the epigeous mushroom genus, *Suillus* (Grubisha et al., 2002).

30.2 SELECTION PRESSURES FOR EVOLUTION OF A HYPOGEOUS HABIT

Evolution progresses through innumerable, random, and nondirected mutations, some of which chance to improve the fitness of an organism to survive or reproduce. Evolution of hypogeous from epigeous fungi would have involved mutations that enabled fungi to better cope with environmental hazards to reproduction or to otherwise improve fitness.

Continental Australia provides clues to the nature of some of these selection pressures. The world's driest continent, even Australia's rain forests are subject to periodic hot spells or drought, it arguably contains the most diverse hypogeous mycota of any of the continents, an estimated 1500 species in over 150 genera, accounting for nearly a third of the world's total (Bougher and Lebel, 2001; Trappe, unpublished data). Claridge et al. (2000a, 2000b) and Jumpponen et al. (2004) have shown that individual Australian species respond in unique ways to an array of habitat variables, but nonetheless form distinct species associations. Furthermore, studies on more than 200 plots in Southeastern Australia indicate that numbers of species and sporocarps of hypogeous taxa often exceed those of epigeous species, especially in relatively dry habitats (Trappe and Claridge, unpublished data). Even in wet habitats and during the Australian wet seasons, when most mushrooms fruit, intervals of warm, dry weather can desiccate newly forming mushrooms to disrupt the current year's spore production. Once the soil is suitably wetted, however, hypogeous species may continue to mature regardless of aboveground weather. Wetting of soil is enhanced where colonies of hypogeous fungi occur, because the holes dug by animals in search of sporocarps during previous fruiting seasons form temporary discontinuities in soil surface water repellency and catch precipitation runoff, leading to preferential water infiltration (Garkaklis et al., 1998). Thus, the established colonies benefit from the diggings by improved water availability precisely at the colony sites. One may infer that the warm, dry weather episodes that often interrupt periods of precipitation in the Australian fruiting season, in concert with the enhanced water availability provided by diggings at colony sites, would favor the reproductive success of hypogeous over epigeous fungi.

Subalpine or continental climates also exemplify environmental hazards that would favor a hypogeous habit. Fungi in such habitats frequently encounter hard frosts during the fruiting season; most fleshy fungi stop producing spores once frozen, but those fruiting belowground are insulated against frost.

These examples are fairly straightforward but do not cover all the possibilities. For example, the coastal fog belt and temperate rain forests of the Pacific Northwestern Coast of the U.S. rarely experience heat, drought, or frost during the fungal fruiting season. Yet hypogeous fungi are common there as well (Trappe, unpublished data). Improvements in

fungal fitness by evolution to a hypogeous habit even in moderate climates may be inferred from the following discussions of mutations in relation to ecology of the fungi.

30.3 MUTATIONS INVOLVED IN SUCCESS OF THE HYPOGEOUS HABIT

Success of evolution from an epigeous to a hypogeous fruiting habit requires a cluster of mutations, and the chances that all necessary mutations happen in appropriate sequence with appropriate timing would seem to be small indeed. We may suppose that the individual mutations have happened innumerable times over millions of years for the occasional required combination to come together by chance. That the mutations have happened so often cannot be evidenced directly, because the genets that did not succeed would often be dead ends that dropped out of the fungal community. The indirect evidence for a high frequency of the required mutations lies in molecular phylogenetic trees of major groups of mycorrhizal fungi. For example, hypogeous members of the Cortinariaceae and Russulaceae have arisen along many branches of the epigeous members of those family trees (Peintner et al., 2001; Miller et al., 2001).

The most obvious mutational change for evolution of an epigeous species to a hypogeous habit is reduction or loss of structures that raise the spore-bearing tissues aboveground. Stems and caps needed to expose those tissues to the air must be modified so that the spore-bearing tissues stay belowground to mature despite adverse weather. Such changes do not necessarily involve many genes (Bruns et al., 1989) and occur in various degrees and combinations. Thus, for example, the hypogeous genera of the Russulales include *Macowanites* still with vestigial cap and stem, but a loculate gleba, and *Gymnomyces*, which has a loculate gleba but lacks cap and stem altogether (Lebel and Trappe, 2000). By reducing or eliminating sterile tissues, these mutations reduce the energy required to form fruiting bodies. This could increase reproductive success when weather truncates the fruiting season or the mycorrhizal host root system restricts the energy supplied to the fungus. Morphological reduction of sporocarps has been usually attended by enclosure of the spore-bearing tissues within a moisture-conserving peridium to further protect the sporocarp from desiccation. Whether these changes involve sequential or linked mutations remains a mystery.

A belowground fruiting habit may protect the sporocarp from environmental assaults, but it improves reproductive success only if effective mechanisms for spore dispersal also evolve. The most common of such mechanisms is production of aromas to attract mycophagists (Parks, 1919). The chemistry of the odors differs from one species to another, some being undetectable by most humans but evident to other mycophagists (Angeletti et al., 1988; Fiecchi, 1988; Pacione et al., 1991). Many mushroom species are fragrant, but little evidence exists to suggest that hypogeous species have evolved more often from fragrant epigeous species than from less fragrant ones.

Some hypogeous fungi have evolved visual cues to attract mycophagists. *Phaeangium lefebvrei*, a desert truffle of the Arabian Peninsula, produces multiple, small fruiting bodies in tight clusters that hump up the soil. Migratory birds spot the humps and scratch the dirt away to reveal the cluster (Alsheikh and Trappe, 1983). The small individual sporocarps in the cluster are a good size for birds to swallow. In New Zealand, *Paurocotylis* and *Weraroa* species are brightly colored (Beever, 1993). The scarlet *Paurocotylis* lifts itself out of the soil as it expands and lies at or on the surface among similarly colored fleshy peduncles of the seeds of overstory *Podocarpus* spp. Birds prize the peduncles and ingest *Paurocotylis* probably by accident while foraging for the peduncles, an apparent

case of mimicry. *Weraroa erythrocephala* has a similar strategy, except its scarlet caps are lifted by a short stipe to just emerge from the soil (Trappe et al., 2001).

Finally, many mushroom genera, for example, *Amanita* and *Cortinarius*, contain species with virulent toxins. It would seem evolutionarily self-defeating for a hypogeous fungus to poison its spore dispersers; indeed, no hypogeous fungi are known to be toxic to humans or other mycophagists. Either the hypogeous relatives of these genera are derived from nontoxic epigeous taxa or they have eliminated toxin production by mutation.

30.4 EFFECT OF PASSAGE THROUGH A DIGESTIVE TRACT ON SPORES

Spores that pass through an animal are subjected to chemical and heat treatments. The spore walls are composed largely of poorly digestable chitin. Epigeous species of ascomycete families such as the Discinaceae have generally unornamented or lightly ornamented hyaline spores. In contrast, a hypogeous genus in that family, *Hydnotrya*, has evolved a thick, heavily pigmented spore ornamentation. This may play no special role in the fitness of *Hydnotrya* species, but it gives the impression of a protective barrier against digestive chemicals. On the other hand, many hypogeous ascomycetes and basidiomycetes have smooth, hyaline, thin-walled spores. Most *Rhizopogon* species, for example, produce minute, delicate-appearing spores that nonetheless remain viable after passage through a gut (Colgan and Claridge, 2002).

Glomus spores extracted from feces of various mammals germinate (Trappe and Maser, 1976; Rothwell and Holt, 1978), and *Glomus*-containing insects, earthworm casts, and mammal feces are effective arbuscular mycorrhizal incoculum (McIlveen and Cole, 1976; Rothwell and Holt, 1978; Ponder, 1980; Reddell and Spain, 1991; McGee and Baczocha, 1994). The same is true of ectomycorrhizal species. Trappe and Maser (1976) observed pregermination structures of *Hymenogaster* spores extracted from rodent feces, and feces containing spores of ectomycorrhizal, hypogeous fungi are effective inoculum for seedlings of suitable hosts (Kotter and Farentinos, 1984a; Lamont et al., 1985; Massicotte et al., 1994; McGee and Baczocha, 1994; Reddell et al., 1997; Colgan and Claridge, 2002).

The evidence for stimulation of spore germination by the heat and chemical treatments of passage through a gut is mixed. In experiments by Lamont et al. (1985), spores of *Mesophellia trabalis* in fecal pellets of a marsupial rat-kangaroo were effective mycorrhizal inoculum, whereas spores of the same species taken directly from fresh sporocarps were not. In contrast, Malajczuk et al. (1987) found that spores taken directly from a sporocarp of *M. trabalis* were effective. Claridge et al. (1992) found that spores of *Andebbia pachythrix* (*Mesophellia pachythrix*) in feces of a rat-kangaroo were effective inoculum, whereas spores taken directly from sporocarps were not. Spores of several *Rhizopogon* spp. are effective inoculum whether in rodent feces or taken directly from sporocarps (Theodorou, 1971; Kotter and Farentinos, 1984a; Castellano and Trappe, 1985; Massicotte et al., 1994; Colgan and Claridge, 2002). Miller (1985) reported that spores of *Tuber* spp. in rodent feces were not effective ectomycorrhizal inoculum unless refrigerated or dried after defecation; i.e., passage through the animal gut did not in itself stimulate germination.

Effects of digestive processes on metabolic activity of spores of a fungal species may differ between animal mycophagists. In feeding trials of *Rhizopogon vinicolor* with flying squirrels, voles, and chipmunks, Colgan and Claridge (2002) compared spores in feces with spores taken directly from sporocarps. Spore metabolic activity was higher after passage

through voles and chipmunks than through flying squirrels or taken fresh from sporocarps, although inoculum effectiveness of spores from the different treatments did not differ.

30.5 ECOLOGICAL CONSIDERATIONS IN EFFECTIVE DISPERSAL AND REPRODUCTION OF SEQUESTRATE FUNGI

At first blush, the sequestrate habit may seem to restrict spore dispersal and reproductive success compared to mushrooms that discharge spores to the air and do not require animal vectors. However, elegant studies of spore dispersal from mushrooms by Allen et al. (1993) call into question the effectiveness of the aerial dispersal strategy. They determined that in a closed-canopy forest, 99% of the spores discharged from mushrooms were deposited on the forest floor within a few meters. Their microclimatological measurements revealed why: airflow at ground level was laminar and slow, even during storms. Consequently, spores discharged from a mushroom dropped rather than being carried upward by turbulence. Only at forest edges where turbulence was strong were significant numbers of spores carried beyond the sampling areas. Some mushroom taxa such as *Russula brevipes* and related species rarely lift their spore-bearing tissues above the humus and instead leave a dense spore print immediately beneath the mushroom cap. In forests with a deep litter layer, only the most robust mushrooms raise their caps out of the litter.

Mushroom-forming fungi are widely distributed and clearly successful, probably due more to profligate spore production than an efficient and effective dispersal system: if billions of spores are produced by a mushroom, chances are some will escape the site of origin. Even then, many may be wasted, alighting where the substrates necessary for survival are lacking. They may also be so dispersed that they rarely form an adequate inoculum load to initiate colonization of a root system or organic matter unless accumulated over time in the soil spore bank.

Given the constraints on aerial spore dispersal from mushrooms, how does mycophagous dispersal of sequestrate species compare? Two strategies have evolved: passage through the digestive tract and release of powdery spores by the mycophagists. The first strategy is the more common: the animal digests the spore-bearing tissue but not the spores, which are defecated. Weathering of the feces may gradually release the spores to be carried into the soil by infiltrating water (Trappe and Maser, 1977). Dung beetles or other insects or coprophilous earthworms may carry the spore-bearing feces into the soil (Christensen, 1980; Claridge et al., 1992).

The second strategy has evolved with only a few genera of hypogeous fungi. At maturity the following produce a powdery spore mass: *Pyrenogaster* and *Radiigera* in the northern hemisphere, the seven genera of the Mesophelliaceae in the southern hemisphere, and *Elaphomyces* and hypogeous *Scleroderma* spp. in both hemispheres. Most of these produce a thick peridium that encloses the powdery spore mass. The peridium is eaten by the animal, which discards the spore powder for release to the wind. Tree climbers such as squirrels often take an excavated sporocarp up a tree to feed, so that the spore powder is released at heights that could result in long-distance transport by wind (Ingold, 1973; Trappe and Maser, 1977). Several genera of the Mesophelliaceae have evolved to a different system. The sporocarp gleba has a central columella or core of dense tissue, with the spore powder produced between the peridium and central core. Claridge et al. (1992) observed that when long-nosed potoroos ate *Mesophellia* sporocarps, they first peeled off the brittle peridium, releasing much of the spore powder to the air and getting much on their paws, noses, whiskers, and fur. The latter spores would be deposited where the animal later

walked or dug for more sporocarps. Spores of *Mesophellia* spp. have been found in feces of various animals (Lamont et al., 1985; Claridge et al., 2001), probably ingested by accident while the animals consumed the sporocarps' inner cores. Such fungi have multifaceted dispersal systems: by moving air; on animal hairs, feet, or noses; and in feces.

The Australian hypogeous genus *Nothocastoreum* at maturity has a dry, brittle, thin peridium enclosing a powdery spore mass. It has never been implicated in mycophagy and would seem to be unpalatable. However, it fruits in large masses in the upper soil. If a mass of mature fruiting bodies is scraped, the peridia rupture and spores puff into the air (Claridge and Trappe, unpublished observation). It may even be that *Nothocastoreum* species produce an aromatic attractant that would fool an animal into digging or scraping the nests of sporocarps in hopes of finding a tasty morsel. Even if the animal did not eat any part of the sporocarps, it would get spores on its feet and lower body fur in addition to releasing many to the air.

At least one hypogeous ascomycete, *Geopora cooperi*, has maintained its forcible spore discharge mechanism, even though the asci are enclosed within the sporocarp chambers (Burdsall, 1965). When excavated and eaten by an animal, however, the spores could be discharged and released to the air through the holes bitten in the peridium by the mycophagist (Laursen and Burdsall, 1976).

Dispersal distance of ingested sequestrate fungal spores is a function of the time required for spores to pass through a digestive tract and the distance traveled by the mycophagist in the interim. Diverse insects tunnel in hypogeous sporocarps to feed or lay eggs (Fogel and Peck, 1975; Hammond and Lawrence, 1989; Bratek et al., 1992). Their overall effectiveness in spore dispersal is unknown, but Ponder (1980) determined that grasshoppers contained effective arbuscular mycorrhizal inoculum, and Bratek et al. (1992) demonstrated by scan electron microscopy (SEM) and light microscopy that insects emerging from mature hypogeous sporocarps carry spores on their bodies or in their guts. Presumably, most would be short-distance dispersers, as would be earthworms (Reddell and Spain, 1991), slugs, and snails (Buller, 1922; McGraw et al., 2002). Small fossorial mammals such as the California red-backed vole (*Clethrionomys californicus*), which spends most of its time belowground, would also not move spores for great distances.

Farther dispersal could be expected of small animals that travel on the ground surface or in the forest canopy, especially species that are not territorial. The northern flying squirrel (*Glaucomys sabrinus*) preferentially eats hypogeous fungi and may have multiple foraging patches of up to a hectare and a home range up to 5 ha (Witt, 1992; Carey et al., 1999). Bandicoots, potoroos, and bettongs in Australia and skunks in North America may travel several hundred meters over 24 h. Animals that travel between forest and deforested areas such as clear-cuts could have a special impact on reinoculation of the latter with spores of sequestrate fungi. MacIntire and Cross (unpublished data) showed that California red-backed voles did not cross the edge from forest into clear-cut, but the opportunistically mycophagist deer mice (*Peromyscus maniculatus*) and chipmunks (*Eutamias* spp.) did where cover and food were adequate in the clear-cut.

Long-distance dispersal of spores may be effected by large, casual mycophagists such as deer, elk, mountain goats, and bear in the northern hemisphere (Cázares and Trappe, 1994; Trappe, unpublished data) or wallabies in Australia (Claridge et al., 2001). However, ability to travel extensively does not necessarily translate into regular extensive travel. For example, wallabies usually stay within territories of less than a half hectare. Still, young males seeking to establish their own territory may travel extensively. After wildfire, wallabies may feed on surviving sporocarps of sequestrate *Mesophellia* spp. (Claridge et al., 2001; Claridge and Trappe, in press), but need to wander to find adequate browse plants; they could thereby transport spores considerable distances.

Mycophagist birds may cover long distances (Alsheikh and Trappe, 1983; Simpson, 1998, 2000). Great grey shrikes prey upon frugivorous lizards in the Canary Islands, ingesting seeds in the lizard guts as they ingest the lizards themselves. By this means the shrikes transport seeds between islands (Moore, 1999). Reason exists to suppose that avian dispersal of fungi would be equally effective. Nonmycophagist raptors may carry their prey for long distances. The northern spotted owl of western North America may traverse territories from about 300 to over 3000 ha (Carey et al., 1992). The mycophagist northern flying squirrel is a primary prey of the owl, which guts it and discards the spore-containing entrails in the forested habitats that it occupies, often far from the point of capture (E. Forsman, personal communication).

30.6 EFFECTIVE PLACEMENT OF INOCULUM

Most mycophagists prefer the habitats in which they forage, so ingested spores are likely to be deposited in habitats suitable for the harvested fungi. Roots of appropriate host plants thus would be available to mycorrhizal fungi. Earthworms, dung beetles, and fossorial animals such as the California red-backed vole would even deposit spores among the roots themselves (Christensen, 1980; Ure and Maser, 1982; Reddell and Spain, 1991). Elimination of mycophagists from a habitat may result in a decline in mycorrhiza formation on plants reliant on spore dispersal by those animals (Gehring et al., 2002).

Strategic spore placement is not the only consideration. Effective inoculation requires an effective inoculum load. Use of spores for inoculation of seedlings in nurseries indicates that a few spores are generally ineffective. Application rates of *Rhizopogon* spores for optimal mycorrhiza formation on seedlings of Pinaceae range from 10,000 to millions per seedling, depending on fungus, host species, and environmental conditions (Theodorou, 1971; Theodorou and Bowen, 1973; Castellano and Trappe, 1985; Massicotte et al., 1994; Colgan and Claridge, 2002). Mean numbers of spores of hypogeous fungi per gram dry weight of feces of tassel-eared squirrels collected in Colorado ranged from ca. 225 million in 1 year to nearly a billion in a second year (Kotter and Farentinos, 1984b). This large point source of spores contrasts with the much dispersed spores of epigeous fungi that escape the immediate vicinity of their parent mushroom. Janos and Sahley (1995) determined that *Glomus* spores comprised up to nearly 10% of the mass of fecal samples of native rodents in Peruvian lowland tropical rain forest. Two of the rodent genera annually passed ca. 300,000 *Sclerocystis* sporocarps and 73 million *Glomus* spores per hectare. Reddell and Spain (1991) found that earthworm casts had a greater spore concentration than adjacent soil in some habitats.

Even widely dispersed spores, however, may accumulate over time in the soil spore bank, a possibly important source of inoculum, especially after stand replacement disturbance (Parke et al., 1984; Miller et al., 1994; Horton et al., 1998; Grogan et al., 2000). Both epigeous and hypogeous fungi might participate in this respect, although their patterns of deposits and longevity of their spores in the soil spore bank may differ (Miller et al., 1994; Bruns et al., 2002).

30.7 EVOLUTIONARY SUCCESS AND GLOBAL WARMING

Today's prevalence of the mycorrhizal habit in terrestrial ecosystems clearly evidences its evolutionary success for both fungi and host plants. Ectomycorrhizae probably originated prior to the Pangaean breakup, which separated Laurasia in the northern hemisphere from

Gondwana in the southern hemisphere. The ocean barriers creating continental drift have precluded much gene flow of fungi, especially hypogeous species, between North and South until relatively recent times (Trappe et al., 2001).

Despite such separation, selection pressures and random mutations independently produced much the same systems of ecosystem resilience and survival in both northern and southern hemispheres: the fungus–plant mycorrhizal association with a subset of animal/sequestrate–fungus/host–plant interdependence (Maser et al., 1978; Emmons, 1982; Perez Calvo et al., 1990; Janos and Sahley, 1995; Claridge et al., 2000a, b; Luoma et al., 2003). The taxa differ between the ecosystems of Gondwanan and Laurasian origin, but the relationships are the same. The nearly universal results of this vastly replicated evolutionary experiment attest to its success.

Past evolution may be a dress rehearsal for ecosystem survival during global warming. Where climate becomes warmer and drier, the hypogeous fungi may be more fit than the epigeous fungi and thereby attain a selective reproductive advantage. The interactions of hypogeous fungi with mycophagists and host trees may then significantly enhance ecosystem sustainability.

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Section 3

Human Impacts on Fungal Communities and Their Function

31

Human Impacts on Biodiversity and Ecosystem Services: An Overview

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31.1 INTRODUCTION

Humans change the composition, structure, and functions of fungal communities in a great variety of ways, as documented in the subsequent chapters of this book. The fungi are only one component of the totality of biological diversity, and one that is far from central to most people's concerns. The purpose of this chapter is to place the specific issues of fungal community change in a broader context of scientific and social concerns about loss of biological diversity and disruption of ecosystem functions, and to identify some of the key research issues that emerge. In so doing, a number of fairly fundamental questions arise. Why should the loss of fungal diversity and disruption of fungal community structure be of concern to society at large? Given that diversity loss will occur, where should we try to draw the line — what level of diversity loss is unacceptable? What are the practical management options for minimizing these impacts and losses?

The protection and sustainable management of the biological diversity of our planet has become a matter of global concern that in 1993 was embodied in the Convention on Biological Diversity (CBD). The general objectives of the CBD (Article 1) are "the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of its benefits arising out of the utilization of genetic resources, including by appropriate transfer of relevant technologies, taking into account all rights to these resources and to technologies, and by appropriate funding." In the CBD (Article 2), *biological diversity* means "the variability among living organisms from all sources, including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species

and of ecosystems." These quotations are taken from the opening chapter of *Global Biodiversity Assessment,* which gives a useful summary of the issues addressed by the convention (Heywood and Baste, 1995). A particular point of reference for this paper is that within the CBD biological diversity is defined with respect to two perspectives: its role in ecosystems and its value to human society. These two dimensions provide the frame for this chapter.

The origins of the agreements of the CBD lie in a variety of concerns about the impacts that human activities have had and are increasingly having on our natural biological resources. While biological scientists have been the main documenters of these changes, concerns about them are shared by a wide constituency of the public. These fears may not always be clearly articulated, and it has to be admitted that biodiversity science is in many respects still in its infancy, but all issues conjoin in general feelings that by reducing the diversity of Earth's biological inheritance, we are in some way diminishing the value of our existence. Some see this issue of value predominantly in economic terms, while for others it is more akin to an issue of ethics. Scientists, including mycologists, may of course adhere to any or all of these concepts of value. But there is one aspect of value that biologists are most qualified to assess — that of the benefits gained from biological functions. This therefore forms a major theme of this chapter.

The most frequent, most substantial, and best-documented effect of human intervention on natural ecosystems is that of food acquisition, whether it is fishing or the use of land for agricultural production. Most of the examples used in this chapter are therefore drawn from the impact of agriculture. Hopefully the points that are made are sufficiently general to also act as a model for other types of human-induced change, such as urbanization, industrialization, and other sources of pollution and land conversion.

31.2 HUMAN IMPACTS ON BIODIVERSITY: THE EXAMPLE OF AGRICULTURE

The factors driving land use change and the type of subsequent agricultural practice are manifold and interactive. Human population growth and the economic and social factors determining agricultural and environmental policies are among the most important and influential ultimate determinants. At a more proximate level, impacts of agriculture on biodiversity are influenced by decisions made at the household and local political or community levels about the crops and livestock to be produced and the methods to be used for their production. These decisions are driven by economic factors of food sufficiency (in subsistence economies), profitability (in commercial farming), or legislative priorities (in the context of subsidized agriculture). The nature and efficiency of agricultural markets, the extent of public and private investment, the quality of institutional support for agriculture, and policies for land use and management are thus all factors that strongly influence the types of agriculture practiced in any given locale.

The apparent homogeneity of agricultural practice in the north temperate zones of America and Western Europe can be misleading. Worldwide there is a huge variation in the types of agricultural system (Beets, 1982). In order to determine the relationship between agricultural practices, diversity, and other features of biological community structure and function, two broad approaches can be taken. The first is that of classical reductionist experimentation — varying single factors, such as introduction of pesticides, and measuring the impact. The second approach, which takes into account interactive effects, is to measure the combined impact of system changes. To carry out comparisons between systems by this approach, it is necessary to have some index of the intensity of

change from the original natural baseline that each system reflects. Such an index is usually related to the concept of agricultural intensification. This can be defined in a number of ways but generally relates to the introduction of methods that enable continuous and increased production on a defined area of land. Factors to be accounted for in such an index may include the fraction of time that land is occupied by crops, the dynamics of primary production, and the quantities of input and output (e.g., see Ruthenberg, 1980; Okigbo and Greenland, 1982).

Agricultural intensification can result in a number of different outcomes. The conventional green revolution type of intensified arable cultivation uses monocrops of high-yielding varieties supported by interventions that substitute industrially produced inputs for biological functions. These include mineral fertilizers for organic inputs, pesticides instead of biological control, and the replacement of biological ploughing by mechanical tillage. In these systems biological diversity is deliberately suppressed in favor of single crops used to simplify management and satisfy market demand. Among the alternatives to this model are those that deliberately retain higher levels of biodiversity. Examples include agroforestry systems, intercropping, rotational farming, cover cropping with legumes, and integrated arable-livestock systems. All of these approaches are more or less closely related to traditional practices of agriculture that still survive in the tropical regions and that are entirely dependent on the mix of biological functions with human knowledge and energy. The values perceived in this utilization of diversity as opposed to the homogeneity of modernized agriculture are multiple and extend beyond those of the market value. They include the desire for multiple products, the spreading of risk, the social and cultural values of certain products, and perceptions of resource conservation and enhanced pest control.

Agricultural intensification reflects changes deliberately introduced by humans, of which the most profound is the choice of which plants and animals should be retained or introduced into the system, i.e., the crops and livestock. Swift et al. (1996) termed this the *planned* diversity and distinguished it from the *associated* diversity — the wide suite of additional organisms, such as the soil biota and the different trophic levels of the pest food webs, that are supported in the system by their association as consumers or symbionts of the primary producers or livestock. The total biological diversity of the intermediate systems referred to above can be very high (Vandermeer et al., 1998). The deliberate maintenance of even a limited diversity of crops and other plants (particularly if trees are included) results in a substantially greater associated diversity in, for example, the aboveground insect population and the belowground invertebrates and microorganisms. Landscapes that include such systems are more likely to conserve biodiversity than those restricted to high-input systems. There is also evidence that mosaics of different systems, including those at different levels of intensification, maintain a higher diversity than monotypic landscapes of any kind, including natural ecosystems on their own (Van Noordwijk et al., 1997).

Stromberger (Chapter 41) documents the changes in fungal communities resulting from agricultural practices. Such changes in the associated diversity are often thought to track those in the plant community; i.e., it has been hypothesized that the plant diversity component of an intensification index would be a highly significant predictor of the associated diversity (Swift and Anderson, 1993). There is, however, little evidence to support or reject this hypothesis, and some that suggests that the soil community in particular may be more functionally resilient than the aboveground biota (Giller et al., 1997).

A key factor in assessing the significance of changes in the diversity of associated organisms, including the fungi, is the assumption that this may cause catastrophic disruption of essential functions, reducing the ability of ecosystems to withstand periods of stress

and leading to undesirable environmental effects. The evidence for such effects is weak. There is a need for further work to quantify the causal relationships between (1) the composition, diversity, and abundance of guilds of associated organisms in agricultural systems; (2) sustained performance of the ecosystem functions of these communities; and (3) the environmental effects associated with diversity change.

31.3 WHY SHOULD SOCIETY CARE? VALUING BIODIVERSITY

The idea, touched on in the introduction, that our concerns about biodiversity loss are connected to concepts of value, has come to constitute a significant component of the agenda for research on biodiversity conservation. Questions of value referred to here are, of course, economic, but they also go beyond economics to ethical, religious, and aesthetic concerns. Taking into account this broader definition, economists recognize four broad categories of value that can be attributed to natural resources: the *direct use* (utilitarian, primary, market) value; the *indirect use* (functional, support) value; the *optional* (future, serependic) value; and the *existence* (nonuse, passive) value (Perrings, 1995). (Note that the terminology varies according to different sources.) All values, whether attributed monetarily or not, are influenced by the economic ideologies and cultural values and preferences of the society within which they are developed. Different societies have different sets of values, and variations in priorities are also found between different sectors within any society.

A *direct use value* can be attributed to an organism or natural resource that is used by humans. In this case, a cash value can be readily assigned as the commercial return that can be obtained from a species or products derived from it. With respect to the fungi, there are but a limited number of examples, most notably edible fungi (truffles may fetch 1500 euros per kg in France) and pharmaceutical and other chemicals derived from fungal genes (e.g., antibiotics such as penicillin).

The *indirect use* or *functional value* of living organisms is that attributed to their role as support systems in preserving the structure and integrity of ecosystems. This describes situations where an organism is not directly used but plays a role in the production of something that is. An important and relatively recent concept that is central to issues of functional value is that of ecosystem goods and services (Daily, 1997), i.e., the recognition that humans benefit in many ways from the functioning of natural ecosystems and that this should be acknowledged by placing values on such services. Ecosystem goods include products derived directly from natural ecosystems (e.g., wild fruits and edible fungi), and thus are adequately dealt with under direct use value. Examples of ecosystem services of benefit to local communities are nutrient cycles that support crop or forest production; the biological control of pests and diseases of plants, animals, and humans; the biological systems that control erosion and retain sediments; and the regulation of hydrological cycles. At a global scale, other services may become important, such as the regulation of the gaseous composition of the atmosphere and thence of the climate. These services, provided by fungi, are explored by Dighton (2003). It is worth noting that although the value, and indeed crucial importance, of such functions may seem obvious to biologists, they are not necessarily accorded such values by other sectors of society. Indeed, conventional economics has often tended to assume that natural resources are renewable and thus can be used at zero or minimum cost. Natural resource economists have over the years developed methods for natural resource accounting by inclusion of the cost of using such resources. By this means, a monetary value is placed on the resource by virtue of its function in providing benefits to society. Costanza et al. (1997) took such

accounting to its logical conclusion by providing an estimate of the total value to humans, in terms of the benefits provided by their services, of the world's ecosystems. Their major conclusion was that this amounted to $33 trillion per year, which is nearly twice the total annual value of global manufactured production.

The *optional value* is closely related to the direct use value in that it refers to the potential, but yet unknown, value of biodiversity to future generations. Where such belief exists, it may be accompanied by a willingness to pay for conservation of the organisms in question. For the fungi, industrial biotechnological products are again the obvious example.

The *existence value* refers to the value that humans place on diversity per se, in its totality or with reference to specific components. This valuation may be inspired by a variety of motives of intellectual, cultural, social, aesthetic, religious, or ethical origin. In some cases, the existence value is linked to some very specific target, such as pandas or rain forest trees. In the case of the fungi, some species (for example, *Amanita muscaria*) have been valued within a religious context. In many cases, it is the very fact of diversity that is appealing. Some people, but sadly not a majority, simply like to see fungi fruiting in variety in the forest in the autumn, irrespective of whether they are edible or doing useful things.

31.4 THE FUNCTIONAL VALUE OF FUNGI

Ecosystem goods and services are of course biologically generated; that is, they are dependent on the efficient maintenance of a wide suite of ecosystem functions — the biochemical and biophysical processes that ensure the productivity, organizational integrity, and perpetuation of the ecosystem. Therefore, if values can be attributed to ecosystem services, they can theoretically also be transferred to the organisms that generate the services. This has formed the basis of many of the attempts to assign value to biodiversity.

The biological processes that generate ecosystem services operate at a variety of scales, ranging from the subcellular through the whole organism and species populations, but it is the effect at the level of the ecosystem that determines their service value (Schulze and Mooney, 1993). At this scale the impacts are the aggregative effect of all the contributive processes and organisms that carry them out. Table 31.1 lists a number of the major categories of ecosystem services referred to above, together with the ecosystem-level functions on which they depend. Listed in the third column are the major groups of organisms that contribute to these functions. In this column the taxonomic groups are aggregated together under headings of "Functional Groups." A functional group can be defined as a set of species that, irrespective of taxonomic relationships, contribute to the same ecosystem function (Swift et al., 2004). This approach has been used in soil biology as a means of focusing attention on function in the diversity debate (Lavelle et al., 1997). It also provides a pragmatic way of organizing and structuring diversity for purposes of analysis.

An example of a functional group that has occupied some significant discussion in the literature is that of ecosystem engineers. These have been definined as organisms that modify ecosystems by constructive activities (Jones et al., 1994; Odling-Smee et al., 2003). The ecosystem engineers that are discussed most frequently are animals such as earthworms or termites that move large amounts of soil and build very obvious structures such as nests or casts (Lavelle, 1996). Some fungi fall into this category, however, by virtue of their activity in binding soil particles together into larger aggregates by the growth of their hyphae (see account by Stromberger, Chapter 41).

The taxonomic categories in Table 31.1 are highly aggregative and thus do not do full justice to the wide diversity of the contribution of the fungi, or indeed any other group.

Nonetheless, even at this level of detail such an accounting surely goes a substantial way toward answering our first question: Why should society be concerned about the loss of fungal diversity? The answer is that the fungi are contributors to almost all the main terrestrial ecosystem services, and in most cases, their role is major and nonsubstitutable.

The most substantial of the fungal functions is their contribution to the processes of decomposition. Decomposition ranks with primary production as the most fundamental of aggregative ecosystem functions (Swift et al., 1979; Swift, 1999). It differs from the latter in being carried out by a very disparate and diverse biota of fungi, bacteria, protists, and invertebrate animals, compared with the relatively conservative disparity and diversity among primary producers. The standing crop equilibrium for both vegetation and soil organic matter is regulated, *inter alia*, by the balance between primary production and decomposition. Fungi can be described as the primary agents of aerobic decomposition because as a group they are almost unique in terms of the range of degradative enzymes they possess, particularly with respect to the primary depolymerizing processes.

As is clear from the frequency with which decomposer organisms are mentioned in Table 31.1, the decomposition function fuels a majority of the ecosystem services in some way. In particular, soil organic matter synthesis is a key process influencing many ecosystem functions.

It is theoretically possible to attribute a fractional value for the fungal contribution to decomposition, soil organic matter synthesis, and ecosystem services. For example, data on the proportion of energy flow that is assignable to the fungal contribution to functions such as decomposition could be converted to the fractional value of the services of interest. Similar calculations could be made for nutrient flows or other parameters. Such data are available and have been estimated from ecosystem simulation models. The presentation of such data as euros rather than calories may indeed be more compelling in persuading society and its decision makers of the value of fungi.

31.5 WHAT LEVEL OF DIVERSITY LOSS CAN OR SHOULD BE TOLERATED?

There is a school of thought that would argue that no biologist should be prepared to tolerate, let alone raise, this question. It can equally be argued, however, that it is irresponsible not to attempt to clarify the scientific issues involved in such a discussion. Many people believe that the perception of an existence value is sufficient reason to maximize the conservation of biodiversity. The setting aside of great tracts of land for conservation is largely a result of such convictions, although it is also argued that such areas are doubly protected if they can also generate income for local communities. What is incumbent on scientists who agree with such policies is to develop and test the appropriate methods for the most effective conservation and for assessing the impact of human interventions, including those with a conservation purpose. Watling reviews some of the necessary approaches with respect to the macrofungi in Chapter 44, and some similar suggestions concerning the microfungi are given in the succeeding section of this chapter. Beyond the ethical reasons for conserving biological diversity, what are the economic and scientific inducements?

There is clearly good commercial reason to conserve the genetic resource base of those species of fungi that have a direct use value. In the end, however, this is likely to be only a very small fraction of total biodiversity. As an example, of the approximately 270,000 known species of flowering plants, only a few hundred have been domesticated for agricultural use, and in the modern world a mere 12 species account for more than

Table 31.1 Major Categories of Ecosystem Goods and Services, the Aggregative Biological Functions on Which They Depend, and Functional Groups of Organisms Responsible

Ecosystem Goods and Services	Ecosystem Functions	Key Functional Groups (Major Taxonomic Representatives)
Ecosystem Goods Including:		
Food	Primary and secondary (herbivore) production	Primary and Secondary Producers (plants, vertebrate herbivores, edible fungi)
Fiber and latex	Primary production and secondary metabolism	Primary Producers (plants)
Pharmaceuticals and agro-chemicals	Secondary metabolism	Primary and Secondary Producers (plants, bacteria, and fungi)
Ecosystem Services Including:		
Nutrient cycling	Primary production Decomposition Mineralization and other elemental transformations	Primary Producers (plants) Decomposers (fungi, bacteria and invertebrates) Elemental Transformers: (bacteria) Root Mutualist Symbionts; (N-fixing bacteria, mycorrhizal fungi)
Regulation of water flow and storage	Soil organic matter synthesis Soil structure regulation – aggregate and pore formation	Decomposers (fungi, bacteria) Ecosystem Engineers (macrofauna, fungi, bacteria)
Regulation of soil and sediment movement	Soil protection Soil organic matter synthesis Soil structure regulation	Primary Producers (plants) Decomposers (fungi, bacteria) Ecosystem engineers (macrofauna, fungi, bacteria)
Regulation of biological populations including diseases and pests*	Plant secondary metabolism Pollination Herbivory Parasitism Predation	Primary Producers (plants) Pollinators (invertebrates, birds, etc.) Herbivores (vertebrates, invertebrates) Parasites (Fungi, Bacteria, Viruses) Predators & Hyper-Parasites (vertebrates, invertebrates, fungi)

Table 31.1 Major Categories of Ecosystem Goods and Services, the Aggregative Biological Functions on Which They Depend, and Functional Groups of Organisms Responsible (Continued)

Ecosystem Goods and Services	Ecosystem Functions	Key Functional Groups (Major Taxonomic Representatives)
Detoxification of chemical or biological hazards including water purification	Decomposition Elemental transformation	Decomposers (fungi, bacteria) Elemental transformers (bacteria)
Regulation of atmospheric composition and climate	Greenhouse gas emission	Decomposers (fungi, bacteria, invertebrates) Elemental Transformers (bacteria) Primary Producers (plants) Herbivores (vertebrates, invertebrates)

* In terms of human values, pathogenic organisms of all kinds have negative connotations, but their importance in regulating natural populations and diminishing the probability of compositional shifts harmful to ecosystem services of various kinds must be included.

Modified from Swift et al., *Agriculture, Ecosystems, and Environment* 104:113–134. With permission.

80% of the annual global production. I am not aware of any comparable data for fungi, but the proportion of the currently known 72,000 species that have been assigned a commercial value must be very low. It is worth noting in passing that commercial value can also be a driver of biodiversity loss and ecosystem degradation if it stimulates over-exploitation or intensive interference with nontarget organisms or their habitat.

Option values provide one of the most persuasive arguments for conservation of diversity. Economists sometimes advocate a precautionary principle whereby if uncertainty exists with respect to the present or future value of a component of diversity, it is better to err on the side of caution and protect it. In the case of fungi, option values provide a case for the investment needed to protect fungal culture collections (Allsopp et al., 1995). But as Watling argues in his chapter, it is far more important to preserve fungal habitats, particularly those that may harbor rare species. The advent of molecular techniques that enable very rapid and efficient screening for useful genes is a two-edged sword. On the one hand, it increases the economic incentive to conserve microbial genomes. On the other, the ability to apply the methods indiscriminately to, for instance, the total microbial genome of soil, can bypass the need to identify the organisms and catalogue the diversity.

The recognition that biological diversity can have indirect use value, i.e., that the fungi and other organisms should be valued because of their importance in maintaining essential ecosystem services, also provides a strong argument for a utilitarian motive for biodiversity conservation. Moreover, it focuses attention beyond nature reserves onto conservation in areas where human interference may be at its maximum — in agricultural landscapes and heavily fished aquatic ecosystems. However, given that these areas will of necessity be ones where impacts on diversity (including extinctions) are inevitable, the

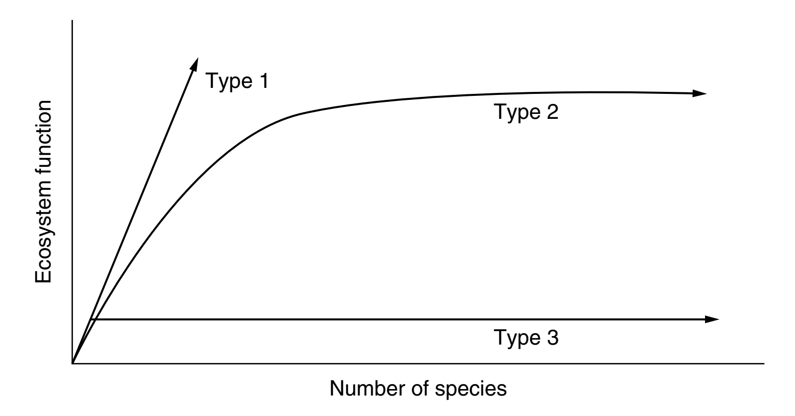


Figure 31.1 Three possible relationships between species diversity and ecosystem functions. (After Vitousek and Hooper, in *Biodiversity and Ecosystem Function*, Schulze, E.D., Mooney, H.A., Eds., Berlin, Springer-Verlag, 1993, pp. 3–14. With permission.)

question is immediately raised as to what proportion of diversity loss should be regarded as an acceptable threshold. This prompts the intriguing scientific quest to determine the relationships between function and diversity. The Occam's Razor approach to this has been the "rivet popper" speculation: if the wing of an aircraft is held in place by a large number of rivets, then it is unlikely to be rendered unsafe by the loss of one. Indeed, such redundancy may be built into the system. But the loss of one is followed by a second and a third, and if this goes on, there will come a time when the wing will fail. So it is hypothesized with species diversity and ecosystem function (see papers in Schulze and Mooney, 1993). How many species are needed and what part of diversity is redundant?

This question has provoked a huge amount of speculation and discussion, as well as a substantial amount of experimentation over the last decade. A great deal of this debate has centered around resolution of a hypothesis proposed by Vitousek and Hooper in 1993. These authors envisaged three ways in which diversity and function might be related (Figure 31.1). Type 1 shows a linear relationship with functional efficiency increasing as a product of ever-increasing diversity (presumably to some unpictured limit determined by external factors). Type 3 shows the absence of any relationship; one species per function satisfies all. It is type 2, however, that Vitousek and Hooper promoted as the likely relationship; functions are enhanced by increasing diversity, but up to relatively small maxima beyond which additional diversity, if present, is redundant.

Much of the subsequent experimentation, mainly on the relationships between plant diversity and system production or nutrient cycling, has supported the type 2 hypothesis, but has been the subject of critical scrutiny and markedly different interpretations (for summaries and references to key papers, see Naeem et al., 2000; Wardle et al., 2000; Naeem, 2000). It is a paradox that we may only be able to understand diversity by simplifying it, and most of the reported experiments adopt this approach by constructing model systems of a manageable number of species. The outcome of such interactions, however, may be quite different from the integrative effects of naturally coevolved and highly interactive communities. For instance, although agricultural systems with a planned diversity of two or more plant species themselves provide tests of the hypothesis, it is clear that they must be regarded with caution as potentially special cases (Swift and Anderson, 1993; Vandermeer et al., 1998). Similarly, model reductionist experiments may not adequately take into account the multiplicity of interactions between plant composition

and the many other factors that can influence ecosystem functions. For instance, variations in the decomposer community have rarely been factored in.

Initial interpretations of the type 2 relationship assumed that the effect was due to the complementarity between functionally distinct species (Vitousek and Hooper, 1993). This could be expressed as showing ecosystem function as dependent on the presence of at least one representative of a number of essential functional groups. In the case of primary production, these are plants with distinct niches because of differing resource capture characteristics (canopy structure, rooting depth, etc.). In some experiments, however, it is clear that one or a few individual species can override the functional group effect through some dominant physiological characteristic (Hooper and Vitousek, 1997).

Decomposition provides an interesting example of the same effects. In natural environments it is common to find that the decomposition of a unit of organic matter (a twig, leaf, insect corpse, etc.) is generally brought about by a significant diversity of fungal species interacting with a similarly diverse community of bacteria and invertebrate animals (Swift, 1976). As proposed above, these organisms can be categorized as different functional groups under the broader rubric of decomposers, for instance, cellulolytic, ligninolytic, and sugar fungi; detritivorous and fungivorous animals. Many of the descriptions of successions on decomposing leaves, twigs, feces, etc., have been portrayed in terms of a sequencing of such distinct functions (e.g., see Chapter 8). The hypothesis could be advanced that the minimum diversity required for decomposition is the presence of one species of each of such functional groups. In direct contrast to this observation of high diversity in many such natural unit communities, it is not uncommon to find decomposition dominated by a single species of common, sufficiently enzyme diverse fungus, such as a basidiomycete. The capacity of such organisms to complete the full process of decomposition of a leaf or branch is easily replicated under experimental conditions.

The answer to the question of how much diversity loss can be tolerated for decomposer fungi is therefore bound to be somewhat equivocal. There are unlikely to be single simple rules like the type 2 functional group hypothesis, but more a variety of relationships dependent on contingencies such as whether and when the resource is invaded by animals. The observation that in many cases several species with the same apparent functional capacity (e.g., cellulolytic ability) may be present provides further complication. Does this imply a redundant level of diversity within functional groups, the existence of niche determinants beyond functional variation, or a simple inability to correctly assign distinctions in functional roles? It is certainly arguable that there is a good deal of redundancy in communities of decomposer fungi and therefore that the loss of many, even the majority of the species, will not result in significant impairment of the decomposition function or the dependent ecosystem services. This is surely a testable hypothesis at the scale of individual resources and in "pot" experiments, although scaling up to the level of the ecosystem is much more challenging. The fungi indeed lend themselves to such experimentation (e.g., see Wicklow and Yocom, 1981). Such experiments would match those on primary producers, but they could be subject to the same criticisms. This is not, however, to suggest that such experiments should not be done. Quite the contrary — the relationship between diversity (together with other community characteristics) and function is clearly of high significance both intellectually and practically.

31.6 MANAGEMENT OF FUNGAL COMMUNITIES

If, as argued but not resolved in the previous sections, there is a link between the diversity, composition, and integrity of biological communities, including fungal communities, and effective ecosystem functioning and service provision, then there is clearly value in

managing ecosystems and biological communities to conserve the communities and optimize the functions. Explicit management of microbial communities with specific functional targets remains, however, a major challenge. While some populations and communities, such as pathogens, have been successfully targeted, other groups, such as decomposers, remain much less accessible to manipulation.

Work on the biological management of soil fertility over the last decade has been based around a recognition that human management of, and impact on, microbial communities can be broadly divided into two categories of intervention: *direct* and *indirect* (Swift, 1998; Brown et al., 2004). Both types of intervention can result in positive or negative effects on biological diversity and community structure and function. *Direct* interventions are those that involve explicit manipulation of a target species or group of species. Fungal examples with positive targets include inoculation with mycorrhiza or the addition of specific decomposer inoculum to compost or soil; with a specific negative target in sight, the use of pesticides or biocontrol agents against symbionts pathogenic to humans or plants and animals of value to humans is one of the most frequent management interventions. The impacts of such practices on nontarget species has been well documented, but the functional consequences are less well understood.

Indirect management operates at the level of the system rather than the organism population. In agriculture such interventions include aspects of cropping system design such as the choice of crops and their spatiotemporal distribution, the management of organic matter and other external inputs, such as fertilizers, and soil management by tillage, irrigation, etc. Also included in this category is the genetic control of ecosystem function by manipulating plant species in terms of resistance to disease, organic matter quality, and root exudation. Although all these interventions have positive targets in mind with respect to crop productivity, they may have positive or negative effects with respect to microbial communities. For instance, higher levels of soil diversity have been consistently observed under conservation tillage compared with conventional mechanized ploughing (e.g., Hendrix et al., 1986). In the positive context of biological management of soil fertility, interventions are designed to serve the concept of enhancing the integrated functioning of the soil community through manipulation of the biotic and physical environment (Brown et al., 2004). Most fundamentally, it is based on a concept of hierarchical management, i.e., that management of the *planned* diversity will influence the nature of the *associated* diversity (Swift, 1999).

If it is accepted that conservation and enhancement of fungal communities are more likely to be achievable by indirect rather than direct management, then a number of implications emerge for the scientific approaches needed for studying fungal communities. First, it is clear that fungal communities should be described and appreciated not in isolation, but as parts of the ecosystems they inhabit, as is also emphasized by Watling (Chapter 44). Interactions with plants, animals, and other microbes strongly influence the composition, organization, and activities of fungal communities, as earlier chapters in this book have abundantly described. Investigation of these interactions becomes important as a means of not just better understanding fungal communities, but also assessing the potential for their management and conservation. Studies of the impact of management on communities and their functions appear to imply the need to assess the total diversity of the community or component guilds. This is rarely a realistic target in microbial ecology. An alternative approach is to develop indicators — species or processes — that have good predictive capacity for diversity or other properties. Molecular techniques now provide the means for indicators of overall diversity, as well as the targeting of specific groups. Indicators may not necessarily be fungal; analysis of the ratios of fungivorous to other nematodes may provide a sensitive indication of change in fungal diversity and abundance that is more cost-effective than determining the fungal data.

Another fundamental problem of microbial community studies is that it is nearly impossible to intervene, experimentally or even observationally, without changing some aspects of diversity, organization, or process. Aggregative functional indicators of ecosystem-level functions may be one way of avoiding such problems, but they require preliminary reductionist investigation of the links between the relationships of diversity and other community properties with functions of ecosystem importance. Better definition of functional groups, with respect to both the fungal participants and those from other taxonomic origins, is likely to be a useful and indeed necessary entry point for such investigations.

31.7 CONCLUSIONS: FUNGAL COMMUNITIES IN AN ECOSYSTEM CONTEXT

Mycologists are concerned about the impacts that human activities are having on fungal communities, including the extinction of species. The main thesis of this chapter is that society at large is unlikely to share these concerns unless it is persuaded of the value of fungi. It is proposed that the most likely way of establishing their value is through their functional importance. The key to this lies in persuading society that ecosystem functions and services must be conserved and sustained by appropriate ecosystem management practices. In the main, this means management of land use and, in particular, vegetational cover. By this means fungal communities will also be conserved — without necessarily making them an explicit target. Although some cord-forming fungi are known to span vast areas, the activities of fungal communities largely take place within scales of a millimeter to a meter. But the need to consider their roles in ecosystems raises the scales of interest to much higher levels, as Morris and Robertson have described in Chapter 1. Moving up the hierarchy of regulation in this way widens the agenda of factors that influence fungal communities and that microbial ecologists must therefore take into consideration.

Governments have typically encouraged land conversion and agricultural intensification in response to conditions of increasing population growth and the consequent demand for higher levels of production of food and other products. Support often comes in the form of set prices for products or subsidy for inputs, although a strong dichotomy exists in how this is applied across the globe. So-called free-market theories have demanded the abandonment of this kind of agricultural support in many developing countries under a variety of structural adjustment and market liberalization reforms, while they continue to be an unmovable element of agricultural policy in the industrial economies of the European Union and the U.S.

Amidst a policy and economic environment that does not acknowledge the importance of managing and conserving agrobiodiversity, farmers, rural communities, scientists, nongovernmental agencies, and the general public have become increasingly aware of the high environmental cost of many intensive high-input agricultural practices. Furthermore, it is now accepted that loss in biodiversity is one of the major factors leading to degradation of ecosystem services and loss of ecosystem resilience. In many countries, however, conflicts have arisen between policies to support biodiversity conservation and ecosystem protection and those of agricultural development. Documentation of the lesser-known components of biodiversity, including the biological populations conserved and managed across the spectrum of agricultural intensification, is an essential component of the information required for assessment of environment–agriculture interactions, as is the evaluation of the impact of agricultural management on the natural resource base. Development of appropriate policy requires, in particular, reconciling the needs for meeting food sufficiency by high levels of agricultural productivity with those for conserving biodiversity

and environmental protection. A major barrier here has been the lack of data on changes in diversity within agricultural landscapes and the assumption that there is necessarily a trade-off between biodiversity and agricultural productivity. There is now, however, growing evidence that farm landscapes can conserve significant levels of biodiversity (Pimentel et al., 1992), particularly where such landscapes are composed of mosaics of land uses of differing degrees of intensification of management (Simberloff and Abele, 1976; Ewel, 1986; Van Noordwijk et al., 1997).

Criteria for managing such landscapes or evaluating them in terms of biodiversity conservation or other features of interest to various sectors of society have yet to be developed (Swift et al., 2004). In some countries policies have been framed with the intention of achieving better integration and to explicitly avoid biodiversity and agriculture being seen as mutually incompatible or competitive. The shift of some parts of the agricultural subsidies in Europe from support for production to support for nature conservation is an encouraging step. However, progress in these respects has been slow. Almost universally, attempts at integrated and sustainable agricultural development are frustrated by lack of an information base that rigorously demonstrates the environmental implications, whether beneficial or detrimental, of agricultural development and the benefits or otherwise to be gained from conservation and management of biodiversity, The contribution to these benefits made by decomposition functions and fungal communities remains one of the largest lacunae. Policy formulation for diversity conservation and management for local, national, and global benefits is dependent on the availability of information, which enables rigorous evaluation of the costs and benefits of different trajectories of development and the reconciliation between them.

The main thesis of this chapter is that the investigation of fungal communities will remain an interesting but academic exercise unless it is undertaken within a context of questioning the function of such communities in ecosystems and in the delivery of ecosystem services. Society will likely only place a high value on the maintenance of fungal diversity when its functional importance is recognized. Grouping organisms into functional groups that are linked to ecosystem services could be a productive way of approaching this. Scientifically, in the words of Morris and Robertson (Chapter 1), it is important to "elucidate the role of specific fungi in contributions to functional processes"; the same agenda taken to a higher aggregative level will enable the better definition of functional niches, which seems essential to the understanding of the links between diversity and function. It is also interesting to speculate what such analysis would tell us of the processes of fungal evolution and ecosystem development. Beyond science, the functional group approach may offer a digestible way of presenting the links between biological diversity and ecosystem services to the public and decision makers.

The interventions of humans into natural ecosystems for their own purposes can have drastic effects on biological communities, including the fungal members. Ironically, these second-order effects may also undermine, through the loss of biological functions, the very benefits that drive the interventions in the first place. In a further irony, the impact of human interventions on communities may offer a useful and accessible framework for investigating and resolving the intriguing web of interactions between biological diversity and ecosystem services.

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32

Oligotrophic Growth of Fungi

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32.1 INTRODUCTION

Oligotrophs are microorganisms that can grow in the presence of low concentrations of nutrients, or even where nutrients appear to be nonexistent. The term *oligotrophy* is generally used to describe the strategy used by microorganisms to grow on low concentrations of organic carbon, more strictly referred to as oligocarbotrophy (Poindexter, 1981). By far, the majority of the literature on microbial oligotrophy relates to bacteria, and some authors have suggested that this growth strategy is restricted to prokaryotes (Fry, 1990). However, as I hope to make clear here, as I have attempted elsewhere (Wainwright, 1993), evidence increasingly shows that many fungi can grow oligotrophically.

The study of bacterial oligotrophy has been hindered by confusion about terminology and the lack of a clear definition of what nutrient level can be considered as constituting oligotrophic conditions; any terms used in a discussion of microbial oligotrophy must, as a result, be clearly defined. In this review, an oligotrophic fungus is defined as one that can grow at low levels of essential nutrients, or even in their apparent absence. Where organic carbon is the nutrient present in low concentration, growth under these conditions is termed *oligocarbotrophy*. A fungus capable of growing in the presence of small amounts of nitrogen will be referred to as an *oligonitrotroph*. This terminology can be further modified to refer to fungal growth in low concentrations of any nutrient; for example, one could refer to oligophosphotrophs and oligoferrotrophs, i.e., fungi that can respectively grow in the presence of low concentrations of phosphorous and iron. Using this terminology, most authors, when discussing bacterial growth under low carbon conditions, have used the term *oligotrophy* as a synonym for *oligocarbotrophy* (Poindexter, 1981; Fry, 1990). In this review, however, the term *oligotrophy* will be used in a generic sense to refer to fungal growth under generalized low-nutrient conditions, rather than with specific reference to organic carbon.

The concentration of organic carbon used to define bacterial oligocarbotrophy has been variously defined, although a concentration of organic carbon of between 1 and 15 g C ml^{-1} has been generally accepted; the lower of these two values was accepted by the Japanese Society for Microbiology (Fry, 1990).

Oligotrophs can be further subdivided into (1) obligate oligotrophs, i.e., those that can grow only at low concentrations of a given nutrient, but will not grow when transferred to media containing high concentrations of that nutrient, and (2) facultative oligotrophs, which grow under both high- and low-nutrient conditions. Although claims for the existence of obligate oligocarbotrophic bacteria have been made (Poindexter, 1981; Fry, 1990), obligately oligotrophic fungi have not yet been reported, a fact that may, however, merely reflect the small number of reports on fungal oligotrophy that have appeared to date.

Are oligotrophs extremophiles? At least one review (Fry, 1990) discusses the growth of oligocarbotrophic bacteria in relation to microbial growth in extreme environments, the obvious implication being that oligotrophic microorganisms are exposed to stress when growing under low-nutrient conditions, with such stress being overcome only when relatively large amounts of nutrients become available. However, it has been argued (Wainwright, 1993) that fungal oligotrophy represents a normal environmental growth response, rather than a reaction to stress.

A wide variety of fungi have been shown to grow oligocarbotrophically on nutrient-free silica gel, including the human pathogenic yeast *Candida* (Wainwright and Al Talhi, 1999) (Table 32.1); oligotrophy is therefore clearly widely distributed among the fungi and is not restricted to an individual species, genus, or group. However, some fungi, mainly wood-decomposing and mycorrhizal species, cannot grow oligotrophically on silica gel and therefore may be regarded as unable to grow as oligotrophs (Table 32.1).

Here, I intend to review the literature on fungal oligotrophy and show how this ability is relevant to the growth of fungi in the natural environment; the relevance of oligotrophic fungi in medicine and industry will also be touched upon (Wainwright et al., 1992, 1993).

Table 32.1 Ability or Lack of Ability of Certain Fungi to Grow Oligocarbotrophically on Silica Gel

Growth	No Growth
Actinomucor	*Clitocybe fragans*
Aspergillus flavus	*Collybia butyracea*
A. niger	*Hypholoma fasciculare*
A. repens	*Lepista nuda*
Candida spp.	*Phanerochaete chrysosporium*
Fusarium oxysporum	*P. velutina*
F. solani	
Gliocladium virens	
Mucor rouxii	
Penicillium chrysogenum	
P. notatum	
Rhizopus stolonifer	
Sordaria fimicola	
Zygorhynchus	

32.2 EVIDENCE FOR FUNGAL OLIGOTROPHY

It can be readily shown that fungi can grow in liquid or on solid media containing otherwise normal concentrations of nutrients, but where carbon and nitrogen are provided at low concentrations (i.e., as oligocarbotrophs and oligonitrotrophs). Fungi also grow in the apparent absence of any nutrient whatsoever, e.g., in what is regarded as ultrapure water, to which no nutrients have been added (Wainwright, 1987, 1988; Parkinson et al., 1989).

Perhaps surprisingly, only a few references to fungal oligotrophy can be found in the early mycological literature. However, Lockwood (1936) noted that fungi often contaminate apparently nutrient-free laboratory reagents, while Castellani (1939) and Stern et al. (1956) showed that fungi can grow in apparently nutrient-free bidistilled water.

Mirocha and Devay (1971) made a seminal contribution to the study of fungal growth under oligotrophic conditions, and in the following year, Tribe and Mabadaje (1972) provided another important contribution to our understanding of how fungi grow on nutrient-free silica gel. More recently, studies aimed at further determining the nature of fungal oligotrophy have been reported (Wainwright and Grayston, 1988; Parkinson et al., 1990, 1991). As yet, however, there are no detailed studies on the ability of fungi to grow as oligonitrotrophs, although there exists an extensive literature, mainly from the early part of the 20th century, that deals with the debate over whether fungi fix atmospheric dinitrogen (Senn, 1928). When reassessed from the viewpoint of oligonitrotrophy, this literature points to the marked ability of fungi to scavenge traces of combined nitrogen from the atmosphere and growth medium.

32.3 ISOLATION AND GROWTH OF OLIGOTROPHIC FUNGI

Silica gel and other nutrient-free solidifying agents can be used to isolate oligotrophic fungi from soils or other natural ecosystems (Parkinson et al., 1989). Where oligocarbotrophs or oligonitrotrophs are sought, otherwise complete silica gel medium lacking a source of either C or N can be used (Parkinson et al., 1989). Fungi readily grow from soil crumbs or other natural substrates onto nutrient-free or low-nutrient gels (Parkinson et al., 1989; Barakah, 1992). As colonies often do not form under these conditions, fungal growth is not always obvious and needs to be confirmed using a microscope. Oligocarbotrophic fungi can also be readily isolated from the air by exposing otherwise complete medium to which no carbon source has been added.

Where exacting oligotrophic conditions are sought, fungi can be cultured using media prepared so as to exclude trace contamination. In such studies, ultrapure chemicals are employed and all glassware (used in preference to plastic containers, which may emit volatile organics) is acid washed and then heated in a muffle furnace in order to destroy all traces of potential organic nutrients (Tribe and Mabadaje, 1972; Parkinson et al., 1989).

32.4 FUNGAL MORPHOLOGY DURING OLIGOTROPHIC GROWTH

Not surprisingly, fungal biomass production under oligotrophic conditions is much smaller than is seen when the fungus is grown copiotrophically (i.e., in nutrient-rich media). Under such low-nutrient conditions, fungi produce very fine hyphae that form fine, mycelial mats (i.e., gossamers). These show numerous anastomoses and float just below the surface of

the medium (Wainwright and Grayston, 1988). Gossamers undoubtedly provide a large surface area, thereby aiding nutrient scavenging from the nutrient-poor medium and atmosphere. Most fungi, including soil isolates, produce spores normally, although not luxuriously, under oligotrophic conditions, and microcycle conidiation is common (Dickinson and Bottomley, 1980; Sheehan and Gochenaur, 1984).

Fungi can grow oligotrophically without the apparent involvement of the lysis and utilization of preformed hyphae. Although such lysis can occasionally be seen, when fungi grow oligotrophically on silica gel (Tribe and Mabadaje, 1972), Parkinson et al. (1989) found that hyphae of *Fusarium oxysporum* growing under these conditions did not lyse, but instead remained intact and full of cytoplasm from the point of inoculation to the actively growing hyphal tip. Because fungi have been seen to grow oligocarbotrophically on silica gel and then transfer to fresh gel on at least 20 occasions (Parkinson et al., 1989), it is clear that they can grow without relying on the lysis and reutilization of preformed biomass; instead, they utilize exogenous nutrient that they obtain by scavenging.

Cytoplasm-free hyphae are, however, often observed when fungi grow under low-nutrient conditions. Dickinson and Bottomley (1980), for example, showed that when spores of *Alternaria* and *Cladosporium* germinate in a nutrient-free solution, the hyphae that are initially formed become evacuated and remain empty. A recent model of fungal growth by Schnurer and Paustian (1986) similarly predicts that fungi produce cytoplasm-free hyphae when growing in soils; cytoplasm can then be translocated where necessary. Such a growth pattern is conservative in terms of energy costs because the formation of cytoplasm is energetically less costly than is the production of fungal wall. Schnurer and Paustian (1986) also calculated that the soils they studied lacked sufficient carbon to support the measured fungal biomass when both cytoplasm and walls were taken into account. Sufficient carbon was present only if production of the latter was assumed. This model is interesting because it suggests that scavenged nutrients would be directed toward the cell wall.

32.5 PHYSIOLOGY OF OLIGOTROPHICALLY GROWING FUNGI

It is likely that many of the physiological characteristics that allow bacteria to grow as oligotrophs will also operate in fungi. Mirocha and Devay (1971) suggest that fungi grow as autotrophs in the apparent absence of organic carbon. They suggest that under these conditions fungi fix CO_2 from the atmosphere by using energy obtained from the oxidation of atmospheric hydrogen (i.e., the knall gas reaction). Although CO_2 fixation by fungi was demonstrated in these studies, no evidence was found for the presence of Calvin cycle enzymes, such as RUBP carboxylase, the enzyme that catalyzes fixation of carbon dioxide, a fact more recently confirmed in the case of *Fusarium oxysporum* grown in the apparent absence of nutrients (Parkinson et al., 1991). By using stripping autoradiography, these workers also confirmed the findings of Mirocha and Devay (1971) that CO_2 is in some way metabolized by fungi when growing under strict oligocarbotrophic conditions, the label being incorporated into hyphae; killed mycelium, on the other hand, did not take up the label, showing that an active process was operating. As the labeled CO_2 could not be removed by acid washing, it was not present on the wall surface in the form of insoluble carbonates or adsorbed CO_2; by partitioning the cell wall components, it was shown that the label was associated with the wall or wall membranes.

The active assimilation of CO_2 by oligocarbotrophically grown fungi could involve either autotrophic or heterotrophic pathways. Autotrophic CO_2 fixation, however, is an

exceedingly energy-demanding process, which is not usually associated with fungi. Mirocha and Devay (1971) pointed out that hydrogen oxidation might act as the source of energy for such autotrophic growth; they cite supporting evidence to show that *Fusarium* spp. possess the necessary hydrogenases.

Parkinson et al. (1991) studied the fate of the carbon assimilated by oligotrophically grown *F. oxysporum.* They found that approximately half of the fixed carbon was released into the medium, while 67% of that remaining in the mycelium was present in the protein fraction, i.e., a carbon distribution pattern that is similar to that found when fungi fix CO_2 anaplerotically. Some 0.68% of fungal cell carbon was derived from CO_2 under oligotrophic conditions. The specific activity of CO_2 assimilation was 295.5 gmol CO_2 g^{-1} dry weight mycelium. The fact that a biomass yield of 0.56 mg C^{-1} was achieved when *F. oxysporum* was grown on oligocarbotrophic medium confirmed that this fungus was able to scavenge airborne carbon sources.

Fungi appear to grow oligotrophically by scavenging nutrients from the atmosphere and fixing CO_2 heterotrophically. As well as acting as a nutrient, CO_2 may have other functions in the fungal cell. Barinova (1962), for example, found that CO_2 speeds up both the swelling and germination of fungal spores and also catalyzes the formation of protein and nucleoprotein polypeptides from amino acids.

It has been suggested that fungi may supplement their energy requirements by oxidizing inorganic ions, such as ammonium or thiosulfate (Wainwright, 1988). Although such chemolithoheterotrophy is relatively common among heterotrophic bacteria, the ability of fungi to use this growth strategy has not yet been confirmed (Wainwright, 1988).

Wainwright and Grayston (1988) provided evidence that fungi use energy gained from thiosulfate oxidation to help them grow oligotrophically, while Jones et al. (1991) found that *F. oxysporum* could oxidize 13% of the thiosulfate provided to sulfate under oligotrophic conditions, this being sufficient to provide energy for 25% of the observed growth. Chemolithoheterotrophic growth by fungi under oligotrophic conditions, although not proven, would obviously be highly advantageous because it would allow a fungus to obtain energy from inorganic oxidations while directing toward biomass production the small amounts of carbon available in oligotrophic environments.

Mirocha and Devay (1971) have also suggested that fungi might gain energy by oxidizing ammonium to nitrite or nitrate when growing oligotrophically. However, because neither of these end products appeared in the growth medium, they concluded that ammonium oxidation did not provide energy for fungal growth under these conditions. It is worth noting, however, that Tolmstoff (1969) found that conidia of *Verticilllum albo-atrum* are able to oxidize ammonium to the equivalent of an amino group by a pathway that is sensitive to inhibitors such as cyanide. Ammonium salts were shown to stimulate respiration by this fungus in proportion to the amount added and the reactions for both ammonium and oxygen. Tolmstoff (1969) concluded that two ammonium ions contribute one electron and one proton to the reduction of an atom of oxygen, leaving an excess of one proton per ion and an amino (NH_2) radical. If fungi can use this system to generate useful energy, then they may be capable of using atmospheric ammonia as both energy and nitrogen sources. The crucial characteristic of oligotrophy, both in bacteria and in fungi, is that organisms using this strategy must use whatever carbon sources or energy-generating systems are available, even if these, when considered individually, produce only small amounts of biomass or energy, the additive effect being sufficient to support growth. Similarly, mixed-substrate growth, where the fungus uses a wide range of carbon and energy sources simultaneously, is likely to be employed during oligotrophic growth.

Recent studies confirm that most filamentous fungi are incapable of fixing dinitrogen. Kostyaev et al. (1983), for example, found that of 138 strains of fungi (representing 58

species from 25 genera) that they tested, none could fix N. Despite this, claims for fungal N fixation, particularly by wood-rotting species such as *Pleurotus*, continue to appear in the literature (Ginterova and Gallon, 1979). It is likely then that because most, if not all, of filamentous fungi are incapable of fixing nitrogen, combined N (mainly as ammonia) will provide the main source of N for oligotrophically growing fungi. A diffusion gradient is presumably set up between the outside of the fungal hyphae and the surrounding air, thereby enabling gaseous nitrogen and carbon to continuously diffuse into the hyphae. By this means, a fungus could effectively scavenge trace amounts of airborne nutrients much in the manner that has been shown by Geller (1983) to occur in bacteria, by a process that has been demonstrated in heterotrophic bacteria.

Much has been written about the physiological and biochemical attributes of oligocarbotrophic bacteria, some of which is likely to be applicable to oligotrophic fungi. Poindexter (1981) listed the physiological characteristics generally possessed by oligotrophic bacteria; these include (1) a high surface:volume ratio; (2) the ability to use metabolic energy for nutrient uptake, with low endogenous metabolic rates; (3) the capacity for continual nutrient uptake; (4) a large proportion of inducible enzymes; (5) uptake systems that are preferentially used for reserve accumulation, so that net synthesis occurs only when rates of uptake and synthesis of metabolic pools become saturated, hence relatively low maximal growth rates; and (6) uptake systems of high affinity with the ability to use several carbon and energy sources simultaneously.

Because aerobic respiration allows for the most efficient energy generation from the dissimilation of scarce organic substrates, it is realistic to suggest that oligotrophs (both bacteria and fungi) should always be strictly oxybiotic respiratory organisms. Despite the fact that many oligotrophic bacteria can grow as anaerobes, it is generally assumed that aerobic metabolism will be selected by microorganisms when growing in oligotrophic environments.

Although coccoid and rod-shaped bacteria grow oligotrophically (by exhibiting a smaller morphology), the mycelial growth form is particularly advantageous for organisms growing in low-nutrient environments, as it provides a high surface:volume ratio that favors an increase in the ability to accumulate nutrients; the thinner the hyphae, the larger the surface area — a fact that explains why the hyphae of oligotrophically growing fungi are so fine. It also seems unlikely that efficient nutrient transport by oligotrophs would involve substrate modification, e.g., substrate phosphorylation. Low substrate specificity and selectivity would be expected to be advantageous for oligotrophs. Finally, when growing oligotrophically, fungi are unlikely to use the scarce carbon sources available to produce secondary metabolites, unless, of course, production of compounds confirms some direct physiological advantage.

It is perhaps stating the obvious to point out that (as a consequence of the above characteristics) oligotrophically growing fungi will not exhibit the same high growth rates produced during growth under nutrient-rich culture conditions.

32.6 UTILIZATION OF GASES AND VOLATILES BY FUNGI

The atmosphere appears to provide most of the nutrients that fungi use to support growth under apparently nutrient-free conditions. The indoor environment particularly contains a diverse range of gases and volatiles that are potential substrates for fungi, most being excreted by plants, animals, and humans or by the combustion of fossil fuels (Johansson, 1978). Humans, for example, excrete C-rich compounds — mainly aliphatic and aromatic hydrocarbons and acetone. Microorganisms also excrete carbon-containing gases, with the

result that microbiology laboratories and growth rooms are likely to be enriched with a diversity of potentially useful airborne-microbial growth substrates.

A diverse range of carbon compounds is also found in the urban atmosphere, including aliphatic C1-C3 hydrocarbons (e.g., methane), monosaturated hydrocarbons (e.g., ethylene), polysaturated hydrocarbons (e.g., benzapyrene), aromatic hydrocarbons (e.g., benzene), halogenated hydrocarbons (halogenated methanes), aldehydes, ketones, and miscellaneous organic compounds such as phenols and polyols.

Volatiles play a role in fungal nutrition in other outdoor environments. Watson et al. (1984), for example, reported that the biological growths occurring in the vicinity of bonded whiskey distillery warehouses in Scotland consist of a mixed fungal culture, which appears to use volatile distillery alcohols as a source of nutrients. Decomposing wood also emits volatiles that attract fungal mycelium (Mowe et al., 1983).

Fungi can use an extremely diverse range of substrates, including carbon monoxide and hydrocarbons (Wainwright, 1988). The wide substrate specificity of fungi in relation to potential airborne carbon nutrients is further indicated by the ability of *Cunninghamella* spp. to use naphthalene, benzo(a)pyrene, benzo(a)anthracene, and methylcholanthrene (Gibson and Subramanian, 1984). Studies by Onoderna et al. (1989) also show that *Scedosporium* sp. can use gaseous hydrocarbons as a sole source of both carbon and energy. As already mentioned, Mirocha and Devay (1971) suggested that hydrogen may act as an energy source to support the growth of oligotrophically growing fungi. Certain bacteria can use hydrogen as an energy source while simultaneously using ethylene as a source of carbon (Slabova and Nikitin, 1986). Both these gases occur in the soil atmosphere and laboratory air and may provide sufficient nutrients for fungal growth under oligotrophic conditions.

The importance of gases and volatiles in fungal nutrition was summarized by Fries (1973) as follows: “To me it seems beyond doubt that the volatile organic substances must be included among the environmental factors which determine the distribution, rate of growth and mode of development of fungi in nature.”

32.7 OLIGOTROPHIC GROWTH OF FUNGI IN WATER AND ACIDS

Stern et al. (1956) showed that fungi are able to grow in apparently nutrient-free bidistilled water, an ability that is obviously relevant to drinking water quality, notably in relation to bottled water. Parkinson et al. (1989) showed that fungi grow in ultrapure water, apparently by scavenging airborne nutrients; fungi can also remain viable in the mycelial state when preserved for long periods in water (Hartung de Capriles et al., 1989).

The medical implications of the fungal contamination of water are highlighted by the fact that various species contaminate water used in hospital dialysis centers (Montagnac et al., 1991). Fungi can also contaminate mini-peak flow meters used to monitor patients with asthma or chronic airflow obstruction (Ayres et al., 1989), although in this case, dilute sputum and exhaled air probably act as the major source of nutrients.

Fungi are commonly found in carbon and nitrogen-free reagents during long-term storage, particularly during storage over long periods at low temperatures (Castellani, 1939). During the late Victorian era, Marshall Ward (1898) made reference to the ability of *Penicillium* species to grow in solutions of 2 to 9% copper sulfate and strong solutions of copper salts, neutralized with ammonia. Growth was associated with the erosion of the surface of copper and bronze. Marshall Ward (1898) concluded by noting “how remarkably resistant this mould *(Penicillium)* is and how little organic matter it needs for life.” Some

years later, Lockwood (1936) reported mold contamination of solutions of sodium thiosulfate, sodium carbonate, ammonium nitrate, potassium nitrate, and phosphoric acid; the contaminants were mainly species of *Aspergillus* and *Penicillium.* Fungi can also be found growing in extreme conditions even in the apparent absence of nutrients; e.g., *Penicillium purpurogenum* has been found growing in phosphoric acid (0.5%). Lockwood (1936) also refers to the isolation of fungi from 95% solutions of copper sulfate and sulfuric acid (0.5%). Such findings have obvious implications for biodeterioration; *Penicillium lilacinum*, for example, can grow and initiate corrosion in nickel-electroplating baths.

32.8 OLIGOTROPHIC GROWTH OF FUNGI ON SURFACES

Fungi occasionally grow on what are usually regarded as inert surfaces, including metals, glass, and electronic equipment (Smith and Nadim, 1983). Such growth is generally associated with high humidity and is therefore particularly common in the tropics, where as Rasmussen et al. (1968) conclude, there is no such thing as a surface that cannot support microbial growth. Animals, including humans and plants, excrete volatiles and gases that can act as nutrients for microorganisms. Microorganisms themselves also excrete volatile nutrients that can be scavenged by other microorganisms. Such products can corrode metals independently, as well as act as nutrients to support microbial corrosion (Rasmussen et al., 1968).

In humid industrial environments, nutrients that are capable of supporting microbial growth include volatile hydrocarbons that are released from machinery, skin and fingerprints, perspiration, and even exhaled cigarette smoke. These substances condense on apparently inert surfaces where they act as a nutrient source for fungi and other microorganisms. A so-called molecular wind has been recognized, occurring on any surface that is subjected to films of condensed liquid. This leads to the entrapment of volatiles, which then evaporate. Substances dissolved in the water film are concentrated, and the equilibrium of the dissolved chemical shifts in favor of the formation of less volatile substances. This is a naturally occurring cycle that eventually causes any exposed tropical surface to develop an ever-thickening layer of substances capable of condensing from the atmosphere (Rasmussen et al., 1968).

The ability of fungi to grow on optical glass, most notably in the humid tropics, has long been recognized. Kerner-Gang (1977), for example, found approximately 100 species of fungi contaminating optical glass, including species of *Aspergillus* (e.g., *A. fumigatus*, *A. glaucus*); *Penicillium* spp. and *Scopulariopsis repens* have been reported by Nagamuttu (1967). Fungi can also grow on the surface of medieval glass in church windows (Tennent, 1980).

The pattern of fungal growth on glass lenses (Nagamuttu, 1967) is essentially identical to that seen when fungi grow on nutrient-free silica gel. In both cases, very fine hyphae are formed, which are frequently connected by anastomoses, resulting in the formation of a network of interconnected fine hyphae. As has already been mentioned, such a growth pattern appears to lead to an increase in surface area and, thereby, more efficient scavenging of substrates from solution or from the atmosphere.

Glass surfaces contaminated with fungi often show signs of pitting, a process that is generally assumed to be caused by the production of organic acids and chelating agents (Richards, 1949). Nagamuttu (1967) showed that nutrient-rich moisture can also condense around fungal hyphae and that these deposits often form as sodium or potassium carbonate, principally syngenite ($K_2SO_4CaSO_4{\cdot}H_20$) (Tennent, 1980). Even where glass is assumed to be clean, it is still often contaminated with small organic particles (Nagamuttu, 1967)

that are capable of supporting microbial growth. Inoue (1988) also described how fungi grow on aluminium, plastics, and electronic components and isolated various fungi from printed circuits and PVC covering electric cables. Similarly, Teitell et al. (1955) noted that fungi often contaminate electrical insulating material, even though growth is not always visible to the naked eye. Mold growth on wire insulation is potentially dangerous because it can lead to an increase in conductance. It is assumed that such growth is supported by scavenged nutrients, rather than by utilization of nutrients in the cable covering.

Silicon wafers can also be contaminated, even when they have been scrupulously cleaned. Photographs of fungal growth on silicon wafers again show the fine mycelial gossamer formation, which is typical of oligotrophic growth.

Fungi, including *Aureobasidium pullulans*, *Cladosporium*, and *Phoma* spp., can also grow on a painted surface without degrading the paint itself (Rains, 1962). Nutrients are obtained from dust, grease, and atmospheric gases and volatiles. Such growth is particularly common where relative humidity exceeds 75%. Fungi can also use paint and its constituents (e.g., linseed oil) as substrates (Ross, 1969). Once a fungus has gained a foothold on a painted surface it tends to trap increasing amounts of dust, grease, and dirt, which act as additional nutrient sources (Rains, 1962; Ross, 1969). Shapiro (1958) reported that when painted surfaces in the Panama Canal Zone were cleaned they often became rapidly contaminated with a surface growth of mildew, particularly associated with washcloth strokes. In these circumstances fungi presumably use the fatty acids contained in soaps and commercial cleaning agents as a source of nutrients; Shapiro (1958), however, showed that fungal growth on painted surfaces is nearly always associated with the accumulation of extraneous organic materials. The ability of fungi to grow oligotrophically by scavenging nutrients from the air explains why materials capable of passing standard fungal resistance tests still occasionally become rapidly contaminated with fungal growth (Smith and Nadim, 1983).

32.9 OLIGOTROPHIC GROWTH OF FUNGI ON ROCK AND STONE

A complex community of microorganisms, including algae, bacteria, fungi, and lichens, can be found on the surface of naturally occurring rocks and stone. Such a mixed culture on stone surfaces is usually thought to depend on the utilization of nutrients released from associated photosynthetic organisms (Ortega-Calvo et al., 1991). However, there is no reason to assume that heterotrophic microorganisms cannot also grow on inert stone and rock surfaces by scavenging nutrients from the air and rainwater. Paine (1932), for example, confirmed that rainwater contains sufficient organic matter to support growth of bacteria, and presumably also fungi, on stone.

Microcolonial fungi commonly inhabit desert rocks, often in the apparent absence of higher organisms. These fungi are metabolically active on the rock surface where they scavenge nutrients from windblown dust (Staley et al., 1982). Fungi and bacteria have also been isolated from weathered sandstones used in historical buildings, including cathedrals (De la Torre et al., 1991; Griffiths et al., 1991).

It is generally assumed that deterioration by stone-dwelling microorganisms results from the production of organic acids (De la Torre et al., 1991). However, Palmer et al. (1991) recently showed that while bacteria and fungi produce organic acids when growing on stone, they were not associated with a sufficiently large reduction in pH to cause weathering. It seems, therefore, that microbial acid production is less important in weathering than is generally assumed.

32.10 FUNGAL OLIGONITROTROPHY AND THE DETERIORATION OF WOOD

Many non-nitrogen-fixing microorganisms (i.e., oligonitrotrophs) can be readily isolated from natural environments using apparently nitrogen-free media and are, as a result, often mistakenly thought to be dinitrogen fixers (Okafor, 1973). The introduction of ^{15}N-tracer and acetylene reduction techniques has conclusively shown that many microorganisms, rather than fixing nitrogen, can scavenge combined nitrogen from the atmosphere. Oligonitrotrophy is particularly important in wood-decomposing fungi. Levi and Cowling (1969) showed that wood-destroying fungi conserve the meager nitrogen supplies by preferentially allocating available nitrogen to metabolic substances that are essential for the efficient utilization of wood substrates. The nitrogen used in these circumstances is believed to be scavenged from the wood, rather than the atmosphere or rainwater. It is also worth noting that carbon- and nitrogen-containing volatiles emitted from wood can stimulate the growth of fungi (Fries, 1973) and that fungi often show a tropic growth response toward wood emitting such volatiles (Mowe et al., 1983).

32.11 OLIGOTROPHIC GROWTH OF FUNGI IN SOILS

The ability of fungi and most other microorganisms to grow in soils is generally thought to be limited by the presence of low levels of available carbon (Smith et al., 1986), with continuous growth occurring only where sufficient available carbon is present, e.g., in the rhizosphere or adjacent to plant residues. The amount of organic carbon available to microorganisms when growing in soils has been variously estimated to be 0.17 to 0.67 m*M*, 0.14 m*M*, and below 8.3 n*M*, i.e., below 100 mg C L^{-1}. Smith et al. (1986) reported a value of 60 to 102 mg of available carbon per kilogram of soil.

Microorganisms are generally thought to lie dormant in soils as spores, or else live off endogenous metabolites while they await the input of fresh nutrients, which then stimulate fresh growth and nutrient depletion. Morita (1988) has recently argued that microbial cells exist in the environment in a state of suspended animation, which he terms metabolic arrest. This view of microbial growth in soils has a long history, and soil microorganisms have traditionally been divided into (1) autocthonous types, which are considered to be true soil inhabitants and thus capable of slow, but continuous, growth in soils, and (2) zymogenous types, which are adapted to rapid growth and substrate utilization, followed by a period of dormancy to survive low-nutrient conditions.

Parkinson et al. (1989) showed that fungi can readily grow from soil particles and plant roots onto nutrient-free agar, an observation that suggests that soils should contain sufficient nutrients to support fungal growth (and microbial growth in general) *in situ*. Barakah (1992) has also shown that fungi can be isolated from soils using capillary pedoscopes containing nutrient-free silica gel, a finding that once again points to the fact that that active mycelial growth of fungi in soils should not be limited by a lack of available organic carbon.

The alternative view that soils contain insufficient available carbon to support continuous fungal/microbial growth is based on calculations of carbon input to soils, the amount of fungal biomass present, and estimates of fungal growth rates. Although it seems likely that such models rely on the input of unreliable data, they certainly appear to ignore the fact that fungi can grow oligotrophically by scavenging carbon from the soil solution or soil atmosphere. While soils, distant from the rhizosphere and residues, clearly do not contain large amounts of available carbon, even small amounts of scavenged substrates can support fungal growth.

Fungi will obviously grow in soils, at a far slower rate than in nutrient-rich media, and will tend to produce fine hyphae and fewer reproductive structures and spores. Sheehan and Gochenaur (1984) have probably provided the best approximation so far to account for how fungi grow in soils. These authors showed that *Penicillium* spores germinate in soils to produce a very fine network of hyphae and also that microcycle conidiation is common.

Fungi have been classified in the past on the basis of the substrates they can utilize; thus, for example, we refer to sugar fungi, cellulose, or lignin decomposers. However, successful soil fungi are likely to be able to use a diverse range of different substrates, on occasion at the same time, i.e., mixed-substrate growth. Species of *Mucor*, for example, can grow oligotrophically despite being generally regarded as sugar fungi and thought to need substantial amounts of simple sugars for growth. Therefore, to group fungi in relation to the utilization of a single substrate is artificial and does not truly reflect the ability of fungi to utilize substrates in nature.

The ability of fungi to efficiently scavenge nutrients is obviously of paramount importance to this view of fungal growth in soils. When fungi grow in soils they tend to produce fine hyphae and numerous chlamydospores and rely upon microcycle conidiation (Park, 1954). By growing in air spaces, soil fungi can efficiently scavenge volatiles or gases or obtain nutrients from the soil solution. Under these conditions, fungi will doubtlessly use a wide variety of nutrients simultaneously and also employ a variety of metabolic strategies to gain energy and anabolic carbon (Wainwright, 1988).

It is essential that, when growing oligotrophically, a fungus should be in no way limited either (1) by substrate specialization or (2) by being obligately oligotrophic. Because in order to survive soil fungi must rapidly exploit any new substrate that enters the system, it comes as no surprise to find that most, if not all, fungi are facultative oligotrophs and can switch from oligotrophy to growth under nutrient-rich conditions without suffering an extended lag phase.

Fungal spores that are susceptible to the inhibitory effects of fungistasis will obviously be prevented from germinating in soils except when available carbon is present to overcome this limitation (Mitchell and Dix, 1975). As a result, a fungus whose spores are subjected to fungistasis will be at a disadvantage, compared with actively growing mycelium, in the race to utilize such nutrient inputs. This is obvious because such a spore will need to germinate and grow into a mycelium before it can begin to exploit the newly available nutrients. An oligotrophically growing fungus (provided it is facultative in this respect), in contrast, already exists in the mycelial state and is therefore in an ideal position to immediately use any nutrients that enter the system. It is worth noting that the concept of fungistasis has been developed using spores that have been grown in high-nutrient media. It may well be that this has produced artifacts and that fungal spores grown under oligotrophic conditions, similar to those found in natural environments, will not be subjected to fungistasis.

32.12 CONCLUSIONS: FUNGAL OLIGOTROPHY IN PERSPECTIVE

In the past, the study of fungi in the environment, particularly soils, has been biased toward a phytocentric approach, centered on the role of fungi as pathogens and as agents of litter degradation. Fungal ecologists have generally uncritically used the physiological principles developed using nutrient-rich media. For the many natural environments that are oligotrophic, such physiological principles are likely to be of dubious value in interpreting the growth of fungi in these environments. In addition, the use of such concepts may be

misleading as it inevitably leads the investigator to assume that a fungus can achieve in nature what it is capable of achieving in nutrient-rich culture. Fungi can participate in all manner of transformations in nutrient-rich culture. For example, they produce antibiotics and organic acids; they also nitrify, oxidize sulfur, and bring about the dissolution of insoluble phosphates (Wainwright, 1988).

Which, if any, of these products are formed or transformations mediated when fungi grow in the natural environment? An example of the confusion that can result from applying physiological principles derived from nutrient-rich cultures to oligotrophic environments is provided by recent studies on the microbial decay of stone. It is generally assumed that stone decay (as well as phosphate solubilization) results from the production of organic acids (e.g., citrate) that are produced in quantity when fungi are grown in nutrient-rich culture. However, as indicated above, Palmer et al. (1991) found that organic acids were not responsible for the *in situ* weathering of stone, a process that instead involves the production of cation-chelating agents.

In the introduction to this chapter it was pointed out that microbial oligotrophy, i.e., with reference to oligocarbotrophy in bacteria, has often been discussed in monographs devoted to the microbiology of extreme environments, the obvious implication being that microbial growth in low-nutrient environments represents a condition of stress. Before discussing the merits of this view, it is necessary to provide a definition of stress. An organism can obviously be regarded as being under stress if it is unable, due mainly to limiting external forces, to achieve the same level of growth, activity, and reproduction that can be achieved in the absence of such limitation. The limitation can be a chemical toxicant (e.g., a heavy metal) or a physical effect (e.g., unfavorable temperature or pH). Many organisms, including fungi, can adapt to such stresses and become tolerant. They then become adapted to an otherwise extreme environment, which results from the existence of similar stress-inducing conditions, and, freed from the limitations imposed by the stress, reach a higher level of growth or reproductive potential than was originally achieved when the organism was susceptible to the stress.

At first sight, a low-nutrient environment might appear to be highly stressful. Under such conditions, a fungus clearly cannot produce the large amounts of biomass typically associated with growth in nutrient-rich media and, as a result, might be regarded as stressed. On the other hand, Foster (1949) has eloquently argued that a fungus shows signs of stress when grown under nutrient-rich conditions; secondary metabolite production, for example, can be seen as a means by which the fungus can cope with excess substrate that the organism never meets in such quantity in nature. The fact that only small amounts of biomass are produced when fungi grow oligotrophically can be regarded erroneously as indicating stress. Such a view, however, is clearly influenced by our experience of growing fungi under nutrient-rich conditions in the laboratory. In fact, the small amount of biomass produced by a fungus growing oligotrophically represents the maximum biomass that can be produced under the prevailing low-nutrient conditions. As a result, the organism can be seen to be in equilibrium with the prevailing nutrient regime in that environment. Should even trace amounts of an essential nutrient (carbon and nitrogen) cease to be available, then the fungus would need to fall back on the utilization of reserves. Once these become used up, the fungus will obviously suffer stress and eventually die.

Equally, stress is imposed if a fungus is grown on a high-nutrient medium and then access to nutrients is denied. This situation can be described as starvation, rather than oligotrophy. These two terms are frequently used as if they were synonymous. However, the term *starvation* is best reserved for the condition when nutrient shortage is imposed following a period of nutrient excess. Under these conditions a fungus is clearly stressed because it can no longer support the metabolism and growth rates that it previously maintained under

high-nutrient conditions. Similarly, a fungus would be stressed if, having grown on a nutrient-rich resource such as wood, this resource is used up; quite clearly, the biomass produced would then no longer be sustainable. In these conditions the older parts of the mycelium must lose viability and die, the younger parts utilizing the products of autolysis to a point where the biomass is sustainable by use of the available nutrients. Whether a fungus exposed to starvation conditions can return to an oligotrophic growth pattern is not clear.

It seems that in the absence of added nutrients, most soil fungi can grow as oligotrophs. Oligotrophy would seem to be the rule then, rather than the exception, among common soil molds, and we have yet to find a common soil fungus that is incapable of growing oligotrophically. On the other hand, this is not so for yeasts, ectotrophic mycorrhizal fungi, and wood-decomposing fungi, which seem incapable of growing on nutrient-free silica gel. The inability of such fungi to grow as oligotrophs, however, may prove not to be a hard and fast rule, especially since the oligotrophic abilities of only a relatively few fungi have been evaluated. However, if this rule does hold, then it is tempting to suggest that organisms that are adapted to growth in the presence of continuous supplies of relatively large amounts of substrates (provided by rotting wood or the direct supply of photosynthate from higher plants) do not grow as oligotrophs simply because they have never had the need to do so. Common soil fungi, in contrast, grow in an environment where carbon is at a premium for long periods, and as a result, they must have become adapted to growth under these conditions by including oligotrophy in their physiology.

If it is true that common soil fungi, but not ectotrophic mycorrhizal fungi and wood-decomposing species, can grow oligotrophically, then we are provided with an ideal opportunity to compare the biochemistry of these two groups to determine what factors make an oligotroph.

Obligate oligotrophy would appear to be distinctly disadvantageous to a soil-inhabiting fungus or other microorganism because in this environment nutrients are in a state of continual flux. Under these conditions, an obligate oligotroph would be limited to low rates of biomass production attuned to low-nutrient supply and so would be unable to respond by growing rapidly when new substrates appear. Obligately oligotrophic fungi, should they exist, will presumably be stressed when exposed to high-nutrient conditions; as a result, they would not appear on nutrient-rich isolation media.

It is worth reemphasizing that many so-called sugar fungi can grow oligotrophically in the absence of added substrate. Rather than being an ecological advantage, restriction to a single substrate limits a fungus, which explains why cellulose-decomposing fungi (e.g., *Fusarium* sp.) can use other substrates and also grow oligotrophically. Mixed-substrate growth and metabolic diversity tend to be the norm, rather than the exception, in microbiology.

Does fungal growth under oligotrophic conditions then represent a stressed or non-stressed condition? The answer is somewhat rhetorical. Where a fungus can grow as oligotroph, then oligotrophy is not a stressful condition; however, if a fungus does not possess the mechanisms that enable it to scavenge nutrients under low-nutrient conditions, then exposure to oligotrophic conditions results in stress, culminating in death. Prior to this terminal stage, the fungus can sporulate or enter some other dormant state, perhaps existing in endogenous metabolism on storage products. Fungi capable of oligotrophic growth, in contrast, do not need to sporulate, except as a means of dispersing their progeny in time and space and as a means of inducing genetic variation. As a result, such fungi need not be exposed to the limitation of fungistasis because they can maintain themselves indefinitely in a hyphal form. Nor does the oligotrophic fungus need to build up large supplies of endogenous reserves, which are typically found when fungi are grown under nutrient-rich conditions.

Finally, an example of how the study of fungal oligotrophy can lead in unusual directions is provided by the work of Wainwright and Falih (1997), who showed that the recently discovered carbon allotrope, buckminsterfullerene, can support fungal growth in the absence of an added, easily utilizable carbon source.

In conclusion, then, oligotrophy among free-living soil fungi appears to be the norm rather than the exception. When fungi grow under these conditions, biomass production and growth rates are attuned to the low level of available nutrients, and as a result, the organism is not stressed. On the other hand, nonoligotrophic fungi will be unable to grow in low-nutrient environments, and should such conditions be imposed, they will suffer stress — a condition that, if not relieved, will eventually lead to their death.

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33

Fungal Communities of Desert Ecosystems: Links to Climate Change

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33.1 INTRODUCTION: CLIMATE AND DESERTS

Fungal dynamics and biodiversity patterns in arid environments are tightly coupled to rainfall patterns, the extent of available moisture, and periods of optimal temperatures that occur together within an arid landscape. To predict the potential impacts of climate change and anthropogenic impacts on fungal biodiversity and activity, one has to first understand how the current abiotic environment in arid landscapes constrains and structures the growth, activity, species interactions, and patterns of abundance for fungal assemblages in these landscapes. Zak et al. (1995) have stated that it is the level of resource heterogeneity coupled with abiotic constraints of moisture and temperature that exists in arid ecosystems that controls much of the fungal dynamics in these systems. Desert environments are considered to exhibit the greatest variability in resources that influence the composition and richness of communities occurring in these ecosystems (Polis, 1991; Whitford, 2002).

All biological activity in arid environments is highly water regulated (Noy-Meir, 1973; Leith, 1975; Zak and Freckman, 1991). Climate has been shown to be the main driver for all primary production in arid systems (Whitford et al., 1987; Allen, 1991; Huxman, 2004), and fungal dynamics are subsequently tightly linked to patterns of moisture availability (Zak et al., 1995) and carbon inputs. As one examines current patterns of fungal activity and species occurrences and develops hypotheses that explain fungal diversity patterns in arid systems, one should be struck by the observation that deserts are characterized by both a strong seasonality in precipitation events and high variability in rainfall totals between years.

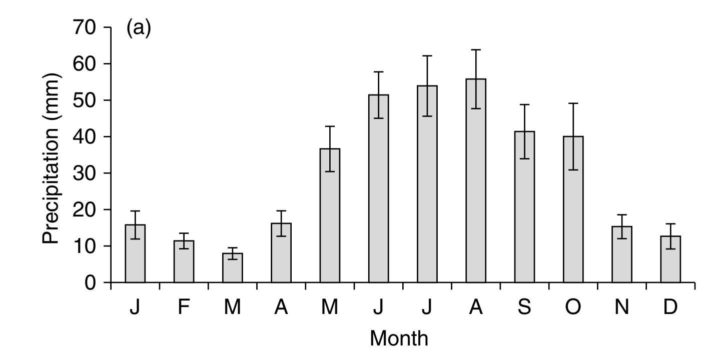
Table 33.1 Variation in the Length of the Period of Maximum Rainfall and Percentage of Total Yearly Precipitation Input for Selected Arid Locations of the World

Locations	Period of Likely Maximum Rainfall	Percentage of Total Yearly Input	Yearly Precipitation Input (mm)
Saharan — Libya	Nov.–Feb.	95	267
Sahel — Senegal	June–Oct.	98	620
Kalahari — South Africa	Feb.–Apr.	56	155
Arabian — Iraq	Nov.–Apr.	95	145
Australian — Kalgoorlie	Feb.–Aug.	73	245
Chihuahuan — Big Bend National Park	June–Oct.	68	330
Sonoran — Santa Rita Experiment Range	Nov.–Mar. June–Sep.	73	300
Monte — South America	Oct.–Mar.	72	197

Some data modified from Whitford, *Ecology of Desert Systems*, New York, Academic Press, 2002.

Most deserts are characterized by a predictable wet season, but not a predictable amount of precipitation during what is considered to be the wet season (Whitford, 2002). For deserts around the world, the length of the wet season can be as short as 2 months (e.g., the Sahara in Libya) or as long as 8 months (Sonoran Desert in Arizona), with the amount of yearly total rainfall falling during the characteristic wet period between 63 and 100% (Table 33.1). In North America, for example, the Chihuahuan Desert in the Big Bend region of far west Texas receives most of its annual rainfall as convectional storms that occur from June through October (Figure 33.1a). The summer rainfall accounts for 68% of the yearly input into this system. During the winter months (November to March), however, when soil temperatures are most near optimum for fungal growth (between 15 and 30°C), the area receives only 16% of the total yearly amount. Although rainfall amounts may be less during the winter months than for the summer in the Chihuahuan Desert, potential evapotranspiration during the winter can be substantially reduced as a consequence of lower air temperatures, thereby increasing the windows of opportunity for fungal activity from the moisture that is received during this period (Sala and Lauenroth, 1982, 1985; Zak et al., 1995). Contrasting the rainfall pattern in the Chihuahuan Desert with the bimodal distribution that is typical for the Sonoran Desert, which is characterized as a winter and summer rainfall dominated system (Figure 33.1b), one begins to appreciate how climate change impacts and shifts in precipitation patterns for arid ecosystems are likely to be system specific and could likely lead to unpredictable impacts on fungi that have become adapted to historical patterns in precipitation patterns and amounts.

While over the long term there is a predictable season of rainfall for desert ecosystems, the amount of rainfall received from year to year can be very unpredictable. For the period 1890 to 2004 for the arid Trans Pecos region of west Texas, which includes the Chihuahuan Desert in the Big Bend region, yearly rainfall totals (Desert Research Institute, 2004) ranged from a minimum of 134 mm to a high of 665 mm, with a medium value of 298 mm (Figure 33.2). Whitford (2002) has stated that the coefficient of precipitation variation may be higher in arid regions than in wet forests and mesic grasslands. However, Conley et al. (1974) report that deserts may not have the largest interannual variation.



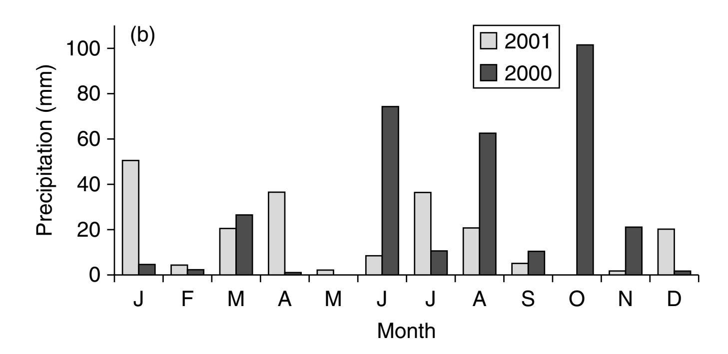


Figure 33.1 Seasonal precipitation patterns for: (a) The Chihuahuan Desert at Big Bend National Park in far-west Texas, and (b) the Santa Rita Experimental Range in the Sonoran Desert of southern Arizona. The Big Bend rainfall data (1986–2003) were obtained from the National Park Service staff at BBNP. For the Sonoran Desert, data for 2000 and 2001 are presented to show the variability that can occur between years. (Data provided by T. Huxman, University of Arizona.)

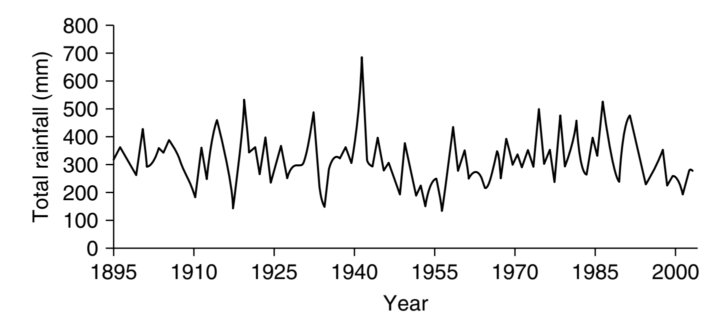


Figure 33.2 Yearly rainfall amounts (mm) from 1895 through 2003 for the Trans Pecos region of Texas, including the Chihuahuan Desert in the Big Bend region of west Texas. Data were obtained from the Western Regional Climate Center, Desert Research Institute website (www. wrcc.dri.edu). To view data for this figure or for other locations in the continental United States, on the wrcc home page, go to Climate Monitoring, USA Divisional Climate Plots, pick the location division you need, and click on Time History Plot # 1.

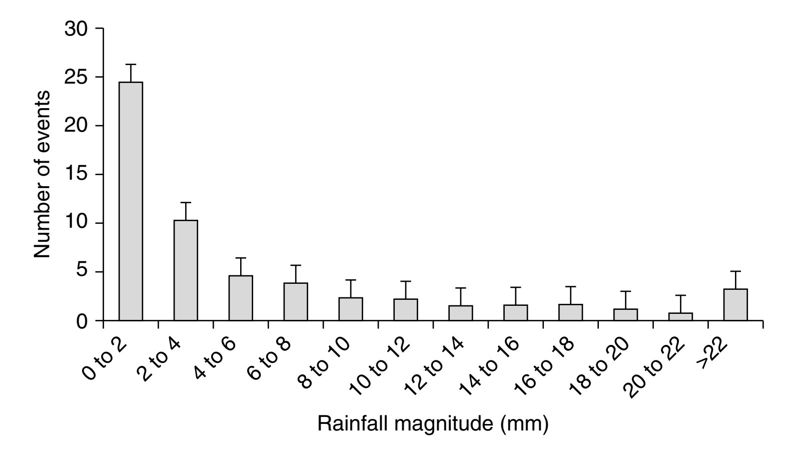


Figure 33.3 The frequency distribution relating the number and size of rainfall events for the Chihuahuan Desert at Big Bend National Park for 1986 through 2003. Values are means +/– SE.

Rather, the constraint on production in arid ecosystems is due to the annual variation in precipitation amounts for desert systems approaching the physiological limits of the biota, while for more mesic landscapes, this threshold is never approached.

Superimposed upon the high coefficient of variation in precipitation amounts between years are the impacts of specific periods of drought that occur in some desert systems. In the Trans Pecos region of far west Texas, multiyear droughts occur about every 20 years (Figure 33.2), with drought severity greater during some periods (e.g., the 1950s) than other (e.g, the 1970s). It is precisely the strong seasonality of precipitation events and the long-term patterns in precipitation that, when coupled to seasonal temperature patterns and high potential evapotranspiration, cause these climate parameters to become the main ecological drivers of fungal activity, fungal biodiversity, and plant productivity in all desert systems. Changes in any of these climate drivers in response to either natural or human-induced climate change will have substantial impacts on the functioning and structure of desert ecosystems and the fungal components of these systems.

Not only is total precipitation amount and yearly variability a factor to consider when modeling the impacts of climate change on fungi in desert systems, but one also has to examine changes in rainfall intensity, duration, and frequency. These aspects of rainfall events determine the biological effectiveness and landscape impacts of precipitation patterns (Whitford, 2002). For the Chihuahuan Desert in Big Bend National Park, the majority of the precipitation events during the year result in small moisture inputs of between a trace and 4 mm (Figure 33.3). The effectiveness of these precipitation events, as mentioned earlier, will vary seasonally, with little impact on plant growth and microbial activity likely during the hot summer months.

Working in the Negev Desert in Israel, Button and Ben-Asher (1983) reported that small rainfall events, <4 mm, had a significant impact on the functioning of this arid system. In a multiyear supplemental irrigation study in a creosotebush bajada area in the northern Chihuahuan Desert in southern New Mexico, Whitford and colleagues (Fisher et al., 1988; Whitford et al., 1988a, 1988b) found that although small rainfall events stimulated the soil microbiota, they were relatively ineffective in promoting plant growth and subsequently increasing carbon availability, which would influence soil fungi. Therefore, changes in rainfall intensities and frequency, as a result of predicted climate change for the Chihuahuan Desert in North America, for example, would likely influence soil micro-

bial responses in these arid ecosystems by altering the duration of moisture windows that allow for fungal activity and by increasing carbon availability.

Potential impacts of climate change on soil fungi in deserts are directly linked to the magnitude of the plant response (alterations in net primary productivity) and subsequent changes in the timing and amounts of carbon inputs into the system. Moreover, if climate change results in increased soil moisture availability during the growing season, as shown by several studies in arid systems around the world (e.g., Charley and Cowling, 1968; Ludwig and Flavill, 1979; Floret et al., 1982; Fisher et al., 1987), soil nitrogen limitations will occur that result in reduced plant productivity despite adequate soil moisture availability.

There is no doubt that precipitation as a driver of ecosystem processes in arid ecosystems is expected to be influenced by global climate change (Houghton et al., 2001; Weltzin et al., 2003). What is unclear at this time is the degree to which changes in precipitation amounts, intensities, and frequencies will alter aboveground net primary productivity (ANPP) in arid ecosystems and the impacts of this landscape-level characteristic on the activity and composition of the soil fungi. While ANPP has been shown to generally increase with greater mean annual precipitation (MAP) sensitivity of ecosystems, changes in MAP differ among ecosystem types (Knapp and Smith, 2001). Huxman et al. (2004) pointed out that the degree of sensitivity of ANPP to changes in precipitation is not well understood but reflects the interactions between vegetation composition and nutrient constraints (Veron et al., 2002). The sensitivity of ANPP to the interannual variation in minimum annual precipitation has been shown to decrease with increasing MAP (Huxman et al., 2004). Of importance to our discussion of the impacts of climate change in arid regions on soil fungi is that arid ecosystems generally exhibit the greatest increase in ANPP with increasing MAP when compared with more mesic ecosystems. As mentioned earlier, however, because water availability has an overriding impact on nutrient cycling in arid landscapes (Schlesinger, 1997), nitrogen availability will likely limit ANPP during extremely wet periods (Smith et al., 1997). Therefore, any consideration of the impacts of climate change on plant liter production and primary productivity in desert landscapes must also include understanding of the counteractive impacts of increasing limitations to ANPP in response to nitrogen availability during periods of adequate soil moisture.

33.2 PREDICTED CLIMATE CHANGE SCENARIOS

Most widely accepted global circulation models predict an increase in mean global precipitation of up to 7% during this century (Houghton et al., 2001). A common prediction from most models, irrespective of the rate of trace gas emissions, is that the increase in precipitation will likely occur in the tropics, mid- and high latitudes (Weltzin et al., 2003), including most of the arid regions around the planet. The models also indicate that the frequency of extreme rainfall events and the intensity of precipitation events will likely increase further for these regions (Easterling et al., 2000). Weltzin et al. (2003) have predicted that shifts in precipitation regimes will have a greater impact on ecosystem dynamics in arid and semiarid regions than the singular or combined effects of rising CO_2 or air temperature. Changes in precipitation were found to substantially change the ecosystem level and individual plant response to elevated CO_2 of an arid ecosystem (Smith et al., 2000).

Focusing on the arid regions of the southwestern U.S. as a point of discussion, the Hadley Climate Model 2 developed by the Hadley Center for Climate Prediction indicates that these arid landscapes will likely see up to a 25% increase in precipitation within the

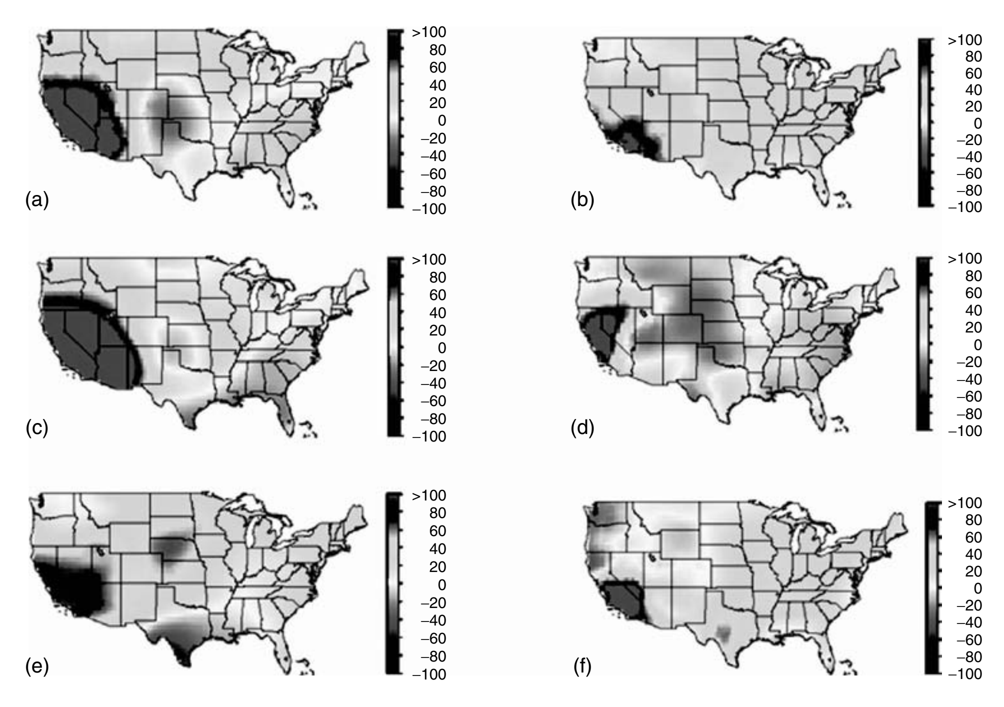


Figure 33.4 **(See color insert following p. 460.)** Percentage change in precipitation amounts for the continental United States as predicted by the Canadian Centre for Climate Modeling and Analysis (CGCM 1) for: (a) annual amounts; (c) December, January, and February (winter); (e) June, July, and August (summer); and by the Hadley Centre for Climate Prediction and Research (HadCM2) for: (b) annual amounts; (d) December, January, and February (winter); and (f) June, July, and August (summer).

next 100 years (Figure 33.4). However, the model also predicts that at the same time, tropospheric warming will increase evaporation rates and potentially increase drought severity despite the increase in precipitation (NAST, 2000).

To understand the potential implications of predicted changes in precipitation on soil fungi in arid regions, such as the southwestern U.S., we first have to understand the current patterns of fungal community structure and organization, functional activity, and the abiotic constraints on fungal dynamics in arid systems. Thus, the remainder of this chapter will discuss our current understanding of fungal diversity in arid ecosystems using the North American deserts and selected studies from the Chihuahuan Desert as examples, provide an assessment of fungal biodiversity for arid regions worldwide when possible, discuss adaptive responses of fungi to arid environments, and lastly consider potential changes in fungal biodiversity in arid ecosystems in response to predicted climate change scenarios.

33.3 FUNGAL DIVERSITY PATTERNS IN DESERTS

33.3.1 Taxonomic Diversity

Early in the last century desert soils were generally considered to be sterile, having few if any microbes that could tolerate the harsh abiotic conditions. Pioneering work by Lipman (1912), Rivkind (1929), and Killian and Feher (1939) clearly demonstrated that indeed many soil microbes could be found in soils from arid regions. In the U.S., the first intensive

investigation of species composition of soil fungi from an arid landscape was conducted by Durrell and Shields (1960) at the Nevada test site. At the same time Rayss and Borut (1958) and Borut (1960) were examining soils from the northern Negev Desert in Israel and Nour (1956) was conducting preliminary assessments of soil fungi from the Sahara. These early investigations in conjunction with subsequent fungal surveys in the Sonoran Desert (Razoni, 1968); high-elevation arid regions of the western U.S. (States, 1978); and soils from Egypt (Moubasher and Moustafa, 1970), the southern desert of Iraq (Al-Doory et al., 1959; Abdullah et al., 1986; Abdullah and Al-Bader, 1990), Kuwait (Moustafa et al., 1976), Libya (Youssef, 1974), and biological crusts in desert grasslands of Utah and Wyoming (States and Christensen, 2001) have described a taxonomically diverse fungal flora for each of these arid regions. Collectively, each of these studies has demonstrated that (1) each location has a characteristic assemblage of soil fungi that is unique to a specific site, (2) dematiaceous and coelomycete fungi with dark hyphae tend to predominate, (3) sterile forms account for high proportions of both taxa and isolates, and (4) generalist species such as *Alternaria* and *Cladosporium* that can be dominant members of fungal assemblages from mesic environments can also be found in these stressed environments. Nicot (1960) attributed the high incidence of black, chlamydosporic fungi in desert soils as an adaptation to ensure persistence under hot, dry conditions.

High soil temperatures for parts of the year are certainly one of the limiting factors influencing the species composition and activity of soil fungi in desert environments. It is not uncommon for the exposed soil surface in the Chihuahuan Desert to reach or exceed 60°C in the afternoon during the summer for several hours (Whitford, 2002). At depths of 15 cm, though, soil temperatures are much cooler and may reach only 30°C for several hours. However, Zak (unpublished data) has observed that for exposed sites in the Chihuahuan Desert at Big Bend National Park, if soils are wet and are subsequently exposed to intense sunlight, such as would occur after thunderstorms in these locations, surface heat is conducted downward into the wet soils, increasing the soil temperatures at 15 cm to almost 45°C.

Several investigators over the years have examined the distribution and occurrence of thermophilic and thermotolerant fungi in arid soils in response to the high soil temperatures that can occur during portions of the year. Survey reports by Moustafa et al. (1976), Abdel-Hafez (1982), Abdullah and Al-Bader (1990), and Mouchacca (1995) have found that thermophilic and thermotolerant fungi can be a significant component of the soil fungal assemblages in arid regions. In particular, 20 thermophilic fungi usually can be enumerated from desert soils if the appropriate incubation temperatures are employed (Table 33.2). These 20 thermophilic taxa are considered to have cosmopolitan distribution (Zak and Wildman, 2004). Zak and Wildman (2004) have suggested that researchers should pay closer attention to habitats in arid regions that accumulate organic matter, such as debris dams (Whitford, 2002) or pack rat middens (Zak et al., 1995), which could attain temperatures into the thermophilic range.

Vegetation patterns in deserts are closely linked to disturbance level, soil texture, soil albedo, precipitation infiltration rates, erodability, and water-holding capacity (Crawford and Gosz, 1986; Whitford, 2002). The structural and functional type of plant that exists in an arid habitat in response to the interaction among the abiotic factors can impact the distribution of soil resources by localizing nutrients, influencing water infiltration rates, increasing soil organic matter levels, and creating favorable environments for microbial activity. These negative-feedback loops increase the spatial heterogeneity of soil nutrients and have led to the increased occurrence of shrub–desert and steppe ecosystems worldwide (Schlesinger et al., 1990, 1996). Mazzarino et al. (1996) found that available nitrogen in the northern Patagonian Desert was related to the vegetation structure of the habitat or

Table 33.2 Selected Thermophilic Fungi That Have Been Isolated from Arid Region Soils

Taxon	Citation for Specific Fungus
Acremonium strictum	Bokhary et al., 1984; Mouchacca, 1995
Chaetomium thermophilum var. *coprophile*	Mouchacca, 1995
C. thermophilium var. *dissitum*	Mouchacca, 1995
Corynascus thermophilus	Mouchacca, 1995
Myriococcum thermophilum	Mouchacca, 1995
Ochroconis gallopava	Horré et al., 1999
Rhizomucor microsporus	Bokhary et al., 1984
Scytalidium thermophilum	Mouchacca, 1995
Talaromyces byssochlamydoides	Mouchacca, 1995
T. emersonii	Bokhary et al., 1984
Thermoascus aegyptiacus	Mouchacca, 1995
T. crustaceus	Bokhary et al., 1984
Thermomyces stellatus	Mouchacca, 1995
Thermophymatospora fibuligera	Udagawa et al., 1986; Mouchacca, 1995
Thielavia heterothallica	Mouchacca, 1995
T. terretris	Mouchacca, 1995

patch. Available nitrogen levels were highest in vegetation patches with a complex mix of shrubs, grasses, and herbs, compared with grass and shrub patches.

Unlike mesic systems, fungal distribution patterns and activities in arid regions are intimately linked to plant distributions and associated patterns of soil nitrogen due to the spatially heterogeneous nature of plant litter and the ability of plants and associated litter to ameliorate the harsh abiotic conditions. Wicklow (1981) had previously indicated that fungal species richness in arid environments is greater than what would be predicted based on consideration of abiotic conditions alone. While low moisture availability and high soil temperatures certainly limit fungal activity, Zak et al. (1995) have stated that it is the high spatial and temporal heterogeneity in carbon availability and other resources that occur in arid ecosystems that may indeed account for the higher than expected fungal species richness that has been observed in arid environments. In arid regions fungi are associated with spatially and temporally heterogeneous substrates that they colonize and grow in during periods when conditions are temporally mesic. These mesic habitats are embedded within the xeric matrix that we perceive as the desert environment, but most fungi either do not grow or may not even survive when conditions become xeric.

In desert environments the distribution of plant litter can be considered as one of the primary determinants of fungal species abundance patterns and species occurrences. On the soil surface, plant litter can accumulate either under scrub canopies, in rodent excavations, in arroyos following rain events (Whitford, 1986, 2002), or through the activities of small mammals, such as wood rats (*Neotoma* sp.) that build complex midden systems around shrubs (Zak et al., 1995; Desjardin et al., 1992) or the banner-tailed kangaroo rats that cache seeds in belowground burrows (Hawkins, 1999). In each of these examples the amount of litter, the litter type, and location of the litter have a crucial role in determining the fungal composition of the fungi colonizing the material.

Table 33.3 Dominant Fungi Associated with Decomposing Cresosotebush Wood (*Larrea tidentata*) That Was Placed on the Soil Surface under the Canopies of the Creosotebush in the Northern Chihuahuan Desert

	Duration in Field[a]		
Fungal Taxa	**1 Year**	**2 Years**	**9 Years**
Coleophoma sp.	92	96	86
Fusarium acuminatum	24	27	14
Alternaria alternata	10	6	9
Philophora richardsiae	8	3	0
Total number of species isolated	22	26	21

[a] Data are percentage frequency of occurrence.

Data modified from Zak et al., *Can. J. Bot*., 73(Suppl. 1):S1407–S1414, 1995.

In general, there is very little known about the patterns of fungal diversity and succession associated with either surface or buried litter in arid regions. The studies cited earlier in this chapter that described fungi from arid regions were all enumerated using dilution plating of soils samples without any connection to soil organic matter particles. For pieces of creosotebush wood placed under the shrub in the Chihuahuan and Sonoran Deserts of North America, Zak et al. (1995) found that the species composition of dominant fungi associated with these wood pieces did not change substantially after 9 years of decomposition (Table 33.3). They suggested that the nutrient quality of the wood coupled with the harsh abiotic conditions limited the number of taxa that could persist in that environment. The dominant fungus from the wood at all sampling times, a *Coleophoma* species, is a coleomycete with dark hyphae supporting the earlier notion concerning the characteristics of fungi in arid environments.

In a more recent and extensive study, Dobranic (2001) enumerated the fungi-colonizing leaf pieces of *Agave lechuguilla* that were placed within five vegetation zones along an elevational gradient (the Pine Canyon Watershed) in the Chihuahuan Desert at Big Bend National Park (Hermann et al., 2000). The Pine Canyon watershed extends 19 km in an easterly direction from the Chisos Mountains and covers approximately 78 km^2 of Chihuahuan Desert landscape in Big Bend National Park. The elevational gradient encompasses five distinct vegetation zones (lowland desert scrub, creosotebush bajada, sotol grassland, closed-canopy oak forest, and high-elevation oak–pine forest). The elevation range is from 793 m at the lowland scrub site to 2098 m in the oak–pine forest. The study by Dobranic (2001) was designed to examine the impacts of vegetation, abiotic constraints, and season on fungal species richness during litter decomposition along the elevational gradient in this Chihuahuan Desert environment. There were substantial differences in the species composition of the fungal assemblages among vegetation zones at all times during the 2-year decomposition study (Table 33.4). Each vegetation zone had a specific group of fungi, though there were fungi common to all zones throughout the study. In particular, a *Coleophoma* sp., the same as that found by Zak et al. (1995) (Table 33.3), and *Microdochium bolleyi* came to dominate the fungal assemblages at various times during decomposition. Seasonal changes in the dominant fungi were also evident. *Coleophoma* sp. was

Table 33.4 Percentage Frequency of Dominant Fungi Isolated from Decomposing *A. lechuguilla* Leaves That Were Placed within Five Vegetation Zones along the Pine Canyon Watershed in the Chihuahuan Desert in Big Bend National Park, TX

	Site				
Taxa	**LS**	**CR**	**SG**	**OF**	**OP**
	6 months				
Alternaria alternata	0	0	15	0	0
Coleophoma sp.	47	56	11	22	6
Exophiala sp.	0.9	33	28	74	0
Fusarium sambucinum	5	0	7	0	7
Microdochium bolleyi	0	<1	9	<1	20
Penicillium crustosum	0	0	12	0	0
Pink yeast	5	0	7	0	7
White yeast	29	5	0	22	20
Total number of species	7	13	13	11	10
	24 months				
Alternaria alternata	0	2	14	1	2
Coleophoma sp.	60	44	6	0	6
Exophilia sp.	5	5	3	0	4
Fusarium acuminatum	4	4	0	0	17
Fusarium oxysporum	0	0	0	36	0
Microdochium bolleyi	7	37	70	10	57
Penicillium citrinum	0	0	0	4	0
Phoma sp. 3	0	0	0	27	0
Phoma sp. 4	20	0	0	0	0
Sterile dark	0	0	0	4	0
White yeast	5	3	0	4	0
Total number of species	7	12	13	16	13

Note: Site codes are as follows: LS = lowland desert scrub; CR = creosotebush bajada; SG = sotol grassland; OF = closed-canopy oak forest; OP = high elevation oak pine forest.

Data modified from Dobranic, Temporal and Spatial Patterns of Fungal Diversity along an Elevational Gradient in an Arid Ecosystem with Implications to Ecosystem Functioning, Ph.D. dissertation, Texas Tech University, Lubbock, 2001.

the most frequently isolated fungus along the watershed during the winter months, while *M. bolleyi* was the dominant member of the litter fungal assemblage from the summer collections. Dobranic (2001) speculated that the differences in species abundances were related to potential differences in ability of these two fungal species to tolerate low litter-moisture conditions, with *M. bolleyi* more likely to tolerate the lower-substrate water potentials during the hot summer months than *Coleophoma*.

As would be expected, differences along the elevational gradient in fungal species composition and frequency of isolation from the agave litter were evident at each sample

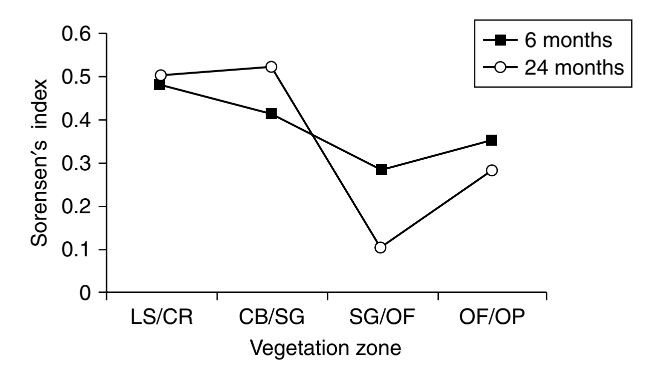


Figure 33.5 Beta diversity of saprophytic fungal assemblages associated with decomposing *Agave lechuguilla* leaves that were placed along the elevational gradient of the Pine Canyon Watershed in the Chihuahuan Desert, Big Bend National Park. Values are Sorensen's Index (quantitative) (Magurran, 1988) between adjacent vegetation zones along the watershed. Site codes are: LS = lowland desert scrub; CR = creosotebush bajada; SG = sotol-grassland; OF = closed canopy oak-forest; OP = high elevation oak–pine forest. Data modified from Dobranic (2001).

time (Dobranic, 2001). Beta diversity between the vegetation zones using the Sorensen Index (quantitative) (Magurran, 1988) was calculated to determine the contribution of each vegetation zone to the overall fungal diversity of the watershed for each of the sample periods during decomposition. Another way to think about beta diversity is to view it as a measure of the degree of similarity between sites (Zak and Willig, 2004). Beta diversity examines the degree of species turnover as one moves from habitat to habitat along an elevational gradient (Southwood, 1978). For the four sample times examined by Dobranic (2001) in the decomposition study (i.e., 6, 12, 18, and 24 months after field placement), beta diversity decreased (Figure 33.5) as one moved up the watershed through the sotol grasslands. These results indicated that the three lower vegetation zones — lowland desert scrub, creosotebush bajada, and sotol grassland — had similar fungal species composition and isolation frequencies for the fungal assemblages on the decomposing lechuguilla leaf litter. Most of the fungal beta diversity for the low portion of the watershed was contained within the lowland scrub vegetation zone. The similarity in fungal species composition within these disparate vegetation zones, although the vegetation type ranged from a low shrub–dominated system through a mid-elevation bunchgrass and sotol (*Dasylirion leiophyllum*), is a result of the constraints imposed on the fungal assemblages in these desert habitats by the high soil temperatures and infrequent and ephemeral moisture windows that are characteristic of these low areas, rather than by vegetation type. However, within the two forested habitats, fungal species composition and frequencies of occurrence changed significantly enough to increase the beta diversity along this component of the watershed. The lower soil surface temperatures and increased rainfall of these higher elevation sites (Zak, unpublished data) were responsible for the shift in beta diversity.

The beta diversity results obtained by Dobranic (2001) for fungi along the Pine Canyon elevational gradient emphasize a significant aspect of the impacts of climate change and shifts in precipitation on desert systems that is often overlooked in general climate models for desert ecosystems. That is, most desert systems usually contain very strong elevational or rainfall gradients of temperature and moisture (MacMahon, 1981; Valentin et al., 1999; Whitford, 2002; Meron et al., 2004) that contribute to the large-scale

patterns of biodiversity across the landscape. Climate change impacts will not only result in local changes within the general desert environments, but predicted changes in precipitation patterns and intensities will likely have their greatest negative impacts on the mid- and high-elevation vegetation zones that are more responsive to temperature and moisture shifts than are the low desert environments. Current efforts by Zak and colleagues to evaluate the impacts of changes in precipitation and intensities on the mid-elevation sotol grassland in Pine Canyon at Big Bend National Park have found that predicted precipitation changes for the region will likely shift the grasslands to a shrub-dominated system by altering nitrogen mineralization patterns associated with the grasses (Nagy, 2003). For desert systems, the ability to predict the sensitivity of vegetation units to climate change impacts and the rate of change is not well understood. Weltzin et al. (2003) have also emphasized the lack of critical information for understanding the impacts of climate change on many types of ecosystems, including deserts.

Within North American desert systems, the distribution of plant litter and the composition and density of the vegetation have been shown to be regulated to various extents by the activities of small mammals, such as kangaroo rats (*Dipodomys* sp.) and wood rats (*Neotoma* sp.) (e.g., Vorhies and Taylor, 1940; Brown and Heske, 1990, Reichman, 1991). The seed foraging, caching, and nest-building activities of these small mammals, especially, play an important role in the distribution of seeds, litter, and subsequently the diversity of saprophytic fungi within most North American deserts. In one of the initial studies of the fungi associated with seed caches and cheek pouches of small mammals in deserts, Frisvad et al. (1987) found a diverse assemblage of fungi, including unique species of *Penicillium*, associated with the banner-tailed kangaroo rat (*D. spectabilis*). Later, Wicklow and Rebar (1988) isolated 30 species of fungi directly from the cheek pouches of *D. spectabilis*. Reichman and Rebar (1985) had previously found that the level of colonization of seeds by fungi influenced the rodent's seed preferences and caching behavior. Recent investigations of seed caches of *D. spectabalis* in the Chihuahuan Desert grasslands in New Mexico (Herrera et al., 1997; Hawkins, 1999) also reported a diverse assemblage of fungi associated with seeds in rodent dens. Furthermore, Herrera et al. (1997) reported that fungal species diversity of food stores in wood rat middens and banner-tailed kangaroo rat dens varied considerably with time and differed among the two rodent genera. They suggested that there was a succession of fungal species that colonized the seed caches of the banner-tailed kangaroo rat and wood rat over time, and that observed patterns in fungal dynamics reflected differences in rainfall patterns between the two sampling areas of the study and animal behavior that influenced restocking rates between the two mammal species.

Frisvad et al. (1987) and Wicklow and Rebar (1988) have suggested that the interaction between the banner-tailed kangaroo rat and the fungi in their seed caches may be mutualistic. The rodents and their dens provide a more amenable environment from that found on or near the soil surface in desert grasslands, carbon resources are readily available from the seeds, and the rodent inoculates the seed with the fungi as the seeds are stored in cheek pouches for transport back to the den (Hawkins, 1999). It has been suggested that the caching behavior of these rodents has indeed selected for fungal strains of more deadly cereal-colonizing fungi that may produce less toxic forms of secondary metabolites (mycotoxins) than are normally associated with these fungi (Wicklow, 1984, 1988). By inoculating the seeds with these less toxigenic strains of fungi from the cheek pouches, the rodent ensures the safety of its food reserves from colonization by potentially more toxigenic fungal isolates.

Changes in precipitation patterns that are predicted to occur for the western U.S. will likely lead to direct influences on caching behaviors of desert rodents and subsequently

alter microclimate conditions in the dens and middens of these small mammals. Alteration in caching dynamics and the microenvironment of the den will subsequently have an impact on the fungi associated with seed caches and the overall fungal diversity for these desert ecosystems. The direction of change for these systems is unclear. However, Herrera et al. (1997) predicted that increased precipitation events in desert systems would likely lead to an increase in fungal diversity associated with these desert rodents. We simply do not know the direction of change in fungal diversity associated with rodent dens or the impact on the population dynamics of the rodents if precipitation in the western U.S. increases as predicted by the Hadley GCM2.

Not only do wood rat middens increase fungal biodiversity in desert environments in which they are located as a consequence of the fungi associated with their cached foodstuffs, but the wood that is used to construct the midden is subsequently colonized by a diverse group of deuteromyctes and basidiomycetes (Anders, 1992; Desjardin et al., 1992; Zak et al., 1995). Early physiological and environmental studies of wood rats by Vorhies (1945) and Rainey (1956) provided data evaluated by Zak et al. (1995) that the construction of the midden provided a mesic environment for the subsequent growth of fungi within the wood pile. Interior temperatures of the midden can be 18°C lower than ambient during the hot summer months. Lee (1963) found that water vapor pressure (dew points) were higher in wood rat middens than for ambient as a consequence of the caching of green vegetation and wood rat respiration. The amelioration of harsh environmental conditions outside the midden would favor extensive fungal growth and fruiting in the middens. In an unusual turn of events, Desjardin et al. (1992) reported the isolation of a new species of *Marasmius*, *M. inaquosi* from paloverde wood collected from the Sonoran Desert in Arizona. They reported that the species is the only known *Marasmius* from desert environments and that the midden building behavior of the wood rat favored the maintenance of this nonarid adapted species in the Sonoran Desert.

In a detailed study of the seasonal occurrence of basidiomycetes associated with wood from wood rat middens found in a mesquite playa fringe on the Jornada LTER site near Las Cruces, NM (Chihuahuan Desert), and from a lowland cresosotebush–saguaro association and an upland paloverde–saguaro association in the Sonoran Desert (the former IBP Silverbell site) near Tucson, AZ, Anders (1992) was able to obtain 280 isolates from plated wood samples over a 2-year period. Eighty-eight basidiomycete isolates were obtained from the Chihuahuan Desert, 91 from Sonoran Desert site 1, and 101 from Sonoran Desert site 2. There were 20 species isolated from middens in the Chihuahuan Desert, 37 from Sonoran site 1, and 24 from Sonoran site 2. While the occurrences of the dominant species were similar among the deserts (Table 33.5), there were substantial differences in the abundances of the taxa within each midden between the two deserts. In the Chihuahuan Desert, middens were dominated by *Peniophora tamaricicola*, while three fungal taxa were isloated with high frequencies from the Sonoran Desert middens (*P. tamaricicola*, *Mycoacia austro-occidentale*, and *Phanerochete omnivorum*). The majority of the fungal isolates obtained by Anders (1992), however, did not produce a basidioma when inoculated onto mesquite wood placed in moist chambers and could not subsequently be identified to a genus or species. Species identifications of basidiomycetes isolated by Anders (1992) from the wood rat middens was achieved primarily by comparison with fungal cultures obtained from tissue or basidiospore from hymenophores obtained from wood in the middens.

The season of maximum abundance of the basidiomycetes isolated from wood rat middens differed for the two deserts. In the Chihuahuan Desert the highest occurrence of basidiomycetes was August through November, while in the Sonoran Desert the months of highest isolations were February and May. Anders (1992) speculated that the patterns

Table 33.5 Daily Growth Rates of Selected Wood Decomposing Basidiomycetes Isolated from Wood Rat Middens from the Chihuahuan and Sonoran Deserts in Response to Seasonal Diurnal Temperature Patterns

	Growth Rates (mm/day)		
Taxa	**5/10°C**	**10/30°C**	**20/40°C**
Chihuahuan Desert			
Hyphoderma pallidum	0.9a	3.6b	5.8c
Peniophora tamaricicola (132)	0.7a	3.4b	5.3c
Peniophora tamaricicola (142)	1.2a	4.1b	6.0c
Isolate 151	0.03a	2.9b	5.1c
Sonoran Desert			
Coprinus sp.	0.0	0.9a	1.7b
Marasmius inaquosi	0.0	0.4a	0.8b
Mycoacia austro-occidentale	0.1a	3.1b	5.5c
Peniophora tamaricicola	0.7a	4.1b	4.8c
Phanerochaete omnivorum	0.0	3.7a	4.5b
Isolate 031	1.0a	1.8b	4.5c

Note: For each isolate ( ), mean growth rates followed by a different letter are significantly different from each other at $p = 0.05$.

Data modified from Anders, Species Composition and Autecology of Basidiomycete Assemblages and Decomposition Dynamics of Wood from Woodrat Middens in the Chihuahuan and Sonoran Deserts, Ph.D. thesis, Texas Tech University, Lubbock, 1992.

of occurrence reflected differences between these two desert systems in patterns of moisture inputs and seasonal patterns of optimal temperatures coupled with rodent behavior. As with the fungal dynamics associated with the banner-tailed kangaroo rat in the Chihuahuan Desert, changes in moisture amounts and input patterns will likely impact the frequencies and species distributions of basidiomycetes associated with the wood rat middens in desert ecosystems in an unknown manner.

33.3.2 Functional Diversity

If climate change results in altered amounts and timing of precipitation events in desert ecosystems as predicted from the recent models for North American deserts, these potential alterations in the length and frequency of moisture windows will likely change ecosystem-level processes that involve fungi. Zak and Visser (1996) had previously emphasized the need to evaluate the linkages between patterns of fungal taxonomic diversity and the functional abilities of the attendant fungi as they influence ecosystem-level processes in order to understand the implications of climate change or other anthropogenic disturbances. Until recently, there has been no effective examination of the linkages between ecosystem-level processes and fungal functional diversity for any ecosystem at the community level, let alone a mechanistic understanding of the consequences of climate change on fungal functional diversity for an arid ecosystem.

Several studies have hinted that specific environments might select for soil fungi that express a greater enzymic or functional diversity than others. Working at the species level, Flanagan (1981) found that the functional capabilities of selected saprophytic fungi isolated from Arctic soils were greater than observed for their more temperate and mesic counterparts. Zak et al. (1995) had emphasized that most Arctic ecosystems, when one uses moisture availability as a criterion, are in fact cold desert environments with moisture limitations as critical as one would find in a traditional arid environment. Zak et al. (1995) stressed that high temporal and spatial heterogeneity in moisture windows is typical for both hot and cold deserts alike and accounts for the greater functional (enzymic) capabilities of the Arctic fungi detected by Flannagan (1981). Working on soil fungi isolated from sites in the Bahamas that were water stressed vs. those that were not, Gochenaur (1975) had also observed that the soil fungi isolated from the xeric (hot and dry) sites expressed greater enzymic versatility than fungal species that were isolated from the more mesic sites.

To facilitate an understanding of functional diversity changes in fungal assemblages during the decomposition of plant litter, Dobranic and Zak (1999) introduced a modification of the Biolog method (Zak et al., 1994) that allows one to determine the abilities of fungi at the community level by using a suite of 95 carbon compounds, from simple carbohydrates to complex polymers. The approach, which they termed the FungiLog method (Dobranic and Zak, 1999), provides for the quantitative examination of the rate of carbon use (i.e., substrate activity) and the number of carbon compounds utilized (substrate richness). Dobranic (2001) subsequently employed the FungiLog approach to evaluate the temporal and spatial dynamics of fungal functional diversity associated with the decomposing *A. lechuguilla* leaves placed along the Pine Canyon elevational gradient in Big Bend National Park, Chihuahuan Desert. The fungal taxonomic diversity patterns observed for this plant litter have been presented in Section 33.3.1. Dobranic (2001) found that the functional diversity of the fungi associated with the decomposing *A. lechuguilla* leaves was significantly influenced by the state of decomposition and the vegetation zone in which the leaf litter was placed. The patterns of substrate activity and substrate richness over time were not consistent among the vegetation zones, but reflected differences in the frequencies and lengths of the moisture windows along the elevational gradient, coupled with elevational and seasonal temperature patterns. The forested areas of the watershed exhibited the greatest fungal functional diversity overall, with no differences observed in fungal functional diversity among the fungal assemblages obtained from the sotol grasslands, creosotebush, and lowland scrub sites. The higher soil temperatures and lower precipitation inputs that characterize the low desert locations along the Pine Canyon Watershed result in the reduction of the functional capabilities of the fungi associated with the decomposing lechuguilla leaves in those locations (Dobranic, 2001).

The impacts of soil and air temperatures coupled with seasonal patterns of optimum soil moisture on the functional diversity of soil fungi in a desert environment were evaluated in a recent study in the Chihuahuan Desert by Sobek (2002). Employing the soil FungiLog method (Sobek and Zak, 2003), Sobek (2002) found that the functional capabilities (substrate richness) of the soil fungi were greater in the low desert locations along the Pine Canyon Watershed during the winter when temperatures were lower, and declined as soil temperatures increased and moisture became limited. At the higher-elevation forested sites, substrate richness of the soil fungal assemblages was greater in the summer when temperatures were optimum and declined during the winter as soils cooled.

Changes in precipitation amounts and frequencies in desert environments not only will influence the size and extent of moisture windows that allow for fungal activity, but

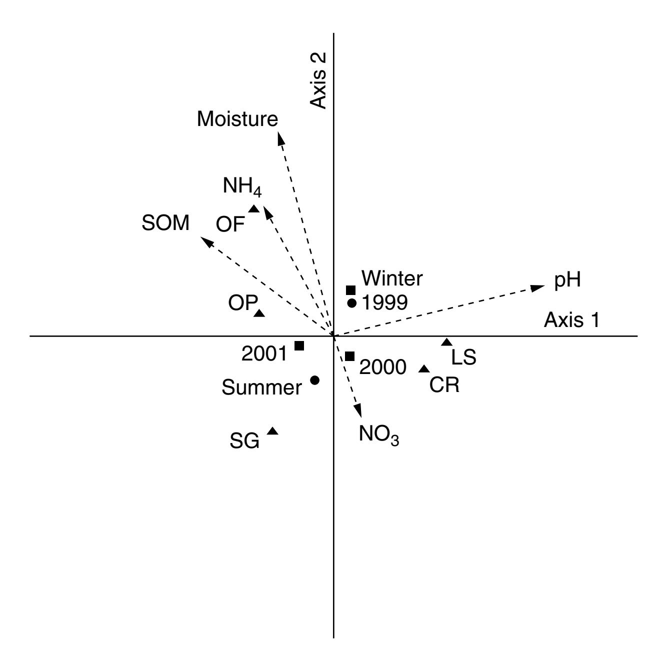


Figure 33.6 The extent to which soil nutrient variables, edaphic factors, soil moisture, and season impact soil fungal functional diversity within the five vegetation zones along the Pine Canyon Watershed in Big Bend National Park, Chihuahuan Desert as visualized using an RPA biplot. Data were grouped by years (1999, 2000, and 2001) and by season, summer (August) and winter (January). Site codes are LS = lowland desert scrub; CR = creosotebush bajada; SG = sotol-grassland; OF = closed canopy oak forest; OP = high elevation oak–pine forest $N0_3$ = extractable NO_3–N; NH_4 = extractable NH_4–N; moisture = soil moisture; SOM = soil organic matter as loss on ignition. Data modified from Sobek and Zak (2003).

also can potentially alter soil nitrogen dynamics, soil pH, and soil organic matter levels over time. Using reduced ranked regression analysis (Jongman et al., 1995; ter Braak and Smilauer, 1998) to evaluate the relationships between environmental variables and soil fungal functional diversity along the Pine Canyon Watershed, Sobek (2002) determined that soil moisture, extractable levels of NH_4-N, pH, and levels of soil organic matter accounted for much of the variation in fungal functional diversity along this Chihuahuan Desert Watershed (Figure 33.6). Soil moisture and levels of NH_4-N were the primary environmental factors influencing levels of fungal functional diversity in the two forested locations, whereas in the sotol grasslands fungal functional diversity was most influenced by conditions that promoted low levels of extractable NH_4-N. Fungal functional diversity of the lowland desert shrub sites was most influenced by environmental conditions that altered levels of extractable NO_3-N in the soil. For the suite of soil nutrient and edaphic parameters included in the analysis by Sobek (2002), differences in rainfall patterns and intensities along the elevational gradient, coupled with seasonal patterns in soil temperatures, as they also regulate nitrogen mineralization rates in this arid landscape, are the likely environmental drivers that account for the observed patterns in fungal functional diversity. The linkages between soil moisture and soil nitrogen dynamics and soil fungal functional diversity that were explored by Sobek (2002) for this Chihuahuan Desert landscape demonstrate that impacts of climate change on fungi in arid systems will be complex.

33.4 FUNGAL ADAPTATIONS

The low numbers of thermophilic fungi that are endemic to arid regions (see Section 33.3) indicate that most soil fungi from arid regions, despite the occurrence of extreme soil temperatures, are mesophilic or thermotolerant. Given the high temperatures that characterize these systems, the lack of widespread thermophily does seem surprising. However, Zak et al. (1995) expressed the view that it is our scale-related biases concerning the distributions of fungi in time and space in arid systems that cause us to make the apparent false assumption that thermophiles should be more widespread in arid regions. We assume that most soil fungi should have heat stress adaptations in order to exist in these arid habitats. Zak et al. (1995) have stated that fungi in deserts avoid the extreme temperatures by either not growing during these periods of high temperatures, escaping through spore production, or at the scale of the mycelium, only colonizing the mesophilic sites that occur in these systems but that change in space and time. We perceive the desert environment as continually xeric and hostile to fungal activity for long periods of time due to our inattentiveness to observational scale.

A critical examination of soil temperatures indicates that, except at the soil surface, readings in the thermophilic range (above 45°C) do not last for more than a couple of hours each day during the hotter portions of the year (Whitford, 2002). For mesophilic fungi, hyphal death at these temperatures depends on the duration of temperatures outside the normal range of growth and the physiological status of the fungus (Griffin, 1994). Exposure to mildly stressful conditions will also lead to increased resistance to more stressful conditions, which have been attributed to the formation of heat-shock proteins (Watson, 1990). The occurrence of heat-shock proteins in most soil fungi from arid regions during the hot portion of the year has not been validated and is only suggested here as one potential mechanism that might account for the lack of thermophilic dominance in these systems.

Anders (1992) evaluated the growth responses of 33 basidiomycete isolates obtained from wood collected from wood rat middens in the Chihuahuan and Sonoran Deserts of southwestern U.S. The temperature study included several isolates of *Coprinus* sp., *Hypoderma pallidum*, *Marasmius inaquosi*, *Mycoacia austro-occidentale*, *Penioophora tamaricicola*, *Phanerochaete omnivorum*, *Sistotrema brinkmanii*, and 13 isolates that could not be identified. Anders reported that optimal growth at single continuous temperatures occurred from 20 to 30°C. Growth rates declined significantly at 35°C, with no growth at continuous exposure to 40°C. Indeed, 32 of the isolates died at exposure to 40°C, with only the *Coprinus* sp. isolates able to recover and subsequently regrow when placed at 25°C. *Marasmius inaquosi* had the broadest temperature optima, with no differences in growth rates from 15 to 30°C. Anders went on to examine the response of these 33 basidiomycetes to diurnal temperature patterns in the lab that mimic the daily temperature changes that occur in these arid systems over the year. She provided a temperature regime of 5/10°C (winter), 10/30°C (spring and fall), and 20/40°C (summer), in which the low temperature was provided for 19 h and the high temperature for 5 h of exposure. Maximum growth rates per day were observed for the 20/40°C diurnal temperature pattern (Table 33.5). Although the single temperature optima for these selected wood-decomposing basidiomycetes was 20 to 30°C, a diurnal temperature regime of 10/30°C could not sustain maximum growth. The growth rates of all fungi at 20/40°C were lower than the growth rates observed for the single temperature study. The results from the studies by Anders (1992) suggest that high temperatures that occur during portions of the year in all desert systems will reduce fungal activity if the duration of the exposure to the high temperatures

is above some critical limit that is unknown for most fungi. The ability of fungi in desert environments to physiologically compensate for detrimental temperatures that occur for only a portion of the day, and then for only a portion of the year, may explain why thermophily is not more common in the arid ecosystem. Thermophily may simply not be an optimum adaptation to a desert environment that is environmentally heterogeneous.

The availability of water for biological activity coupled with seasonal extremes in temperatures does regulate the activity and patterns of fungal species occurrence in desert environments. Often, precipitation patterns that create optimum moisture windows for fungal growth and development coincide with high air and soil temperatures, which subsequently restricts fungal activity and determines the composition of fungal assemblages for particular substrates, such as wood on the soil surface. Fungi are able to tolerate reduced water activity (a_w) in soils and organic substrates when other critical environmental conditions (e.g., pH, temperature, osmotic concentration) are near optimum (Corry, 1987). However, the ability of fungi to tolerate a broader range of pH and temperatures in the environment is restricted as water activity is reduced. Corry reported that some xerotolerant yeasts could not grow at high levels of a_w when culture temperatures were elevated. Alternatively, the growth of the yeast *Zygosaccharomyces rouxii* at 40°C only occurred when the growth medium contained either 3 to 4% NaCl or more than 40% sugar.

Conditions that promote the germination of conidia and other spore forms are frequently different from those for mycelial growth. Ayerst (1969) observed that the germination of *Aspergillus* conidia occurred at high temperatures across a wide range of a_w, with little or no mycelial growth apparent across this range. These temperature–a_w interactions should control colonization rates of plant litters in arid systems and could account for seasonal differences in the composition and densities of fungal assemblages that have been followed on decomposing litter in desert environments.

33.5 POTENTIAL IMPACTS OF CLIMATE CHANGE

The trajectory and extent of change in litter and soil fungal dynamics in arid systems to predicted climate changes in precipitation patterns and intensities and temperatures will depend to a large extent on the rates of ecosystem-level shifts in vegetation and soil nutrient dynamics that are likely to occur concomitantly. Changes in global and regional precipitation patterns are predicted to have significant consequences on plant dynamics, soil fungi, and attendant ecosystem-level processes (Ernest et al., 2000, Shaver et al., 2000; Staddon et al., 2003). For the western U.S. the two major global change models (Hadley GCM2 and CGM1 — Canadian Center for Climate Modeling) suggest that these arid regions may see a 25 to almost a 100% change in precipitation over the next 100 years, with the two models differing in the timing and extent of precipitation changes. Neilson and Drapek (1998) have predicted a substantial greening occurring across wide areas of the arid southwestern U.S. as a result of increases in the density and relative production of C_4 grasses in response to precipitation increases that are predicted under certain climate change scenarios. If the results from the Pine Canyon Watershed studies in the Chihuahuan Desert (Dobranic, 2001; Sobek, 2002; Sobek and Zak, 2003; Nagy, 2003) are indicative of fungal responses to moisture availability and soil temperatures for other arid regions, predicated changes in the amounts and timing of precipitation and subsequent shifts in soil temperatures will lead to alterations in fungal species occurrences, functional diversity, and subsequent ecosystem-level dynamics in complex ways.

Our ability to understand and interpret the level of complexity that governs arid ecosystem response to climate change is hindered by the lack of long-term manipulative

experiments within a variety of arid ecosystems that specifically focus on the rates of change that will occur in plant productivity and species composition, fungal species occurrences and activity, and soil processes in response to precipitation and temperature changes in these systems. Weltzin et al. (2003) have discussed the importance of developing such a network of precipitation manipulation research sites in arid regions of the southwestern U.S. to begin to address issues of climate change impacts in a coordinated manner. Precip net, a national network for precipitation and ecosystem change interdisciplinary research, was recently organized (see homepage of Dr. Michael Loik, UC Santa Cruz: people.ucsc.edu/~mloik/) to help foster and promote the coordination of climate change research efforts across the arid regions of the U.S. and to assist in the dissemination of information.

When one integrates the limited fungal data collected from arid ecosystems with the results from precipitation manipulation studies from desert regions, there is no doubt that fungal assemblages in arid systems will respond to shifts in precipitation patterns and amounts, soil temperatures, and the interactions between moisture and temperature as a consequence of direct impacts of these environmental variables on spore germination and mycelial growth, and through modifications of plant productivity and species composition. For now, the rates of change in fungal dynamics that may occur in desert shrub-dominated systems vs. grasslands, for example, and the stability of vegetation types under predicted climate change scenarios are uncertain. Our challenge over the next decade will be to develop manipulative field experiments in a variety of arid ecosystems that will allow us to ascertain the magnitude of the response of fungal activity in arid ecosystems to climate change and the rates at which fungal species composition and diversity will likely be altered.

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34

Symbiotic Lifestyle Expression by Fungal Endophytes and the Adaptation of Plants to Stress: Unraveling the Complexities of Intimacy

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34.1 INTRODUCTION

The fossil record indicates that fungal symbionts have been associated with plants since the Ordovician period (approximately 400 million years ago), when plants first became established on land (Pirozynski and Malloch, 1975; Simon et al., 1993; Remy et al., 1994; Redecker et al., 2000). Transitioning from aquatic to terrestrial habitats likely presented plants with new stresses, including periods of desiccation. Because symbiotic fungi are known to confer drought tolerance to plants (Read and Camp, 1986; Bacon, 1993), it has been suggested that fungal symbiosis was involved with or responsible for the establishment of land plants (Pirozynski and Malloch, 1975). Symbiosis was first defined by De Bary in 1879, and since that time, all plants in natural ecosystems have been found to be colonized with fungal and bacterial symbionts. It is clear that individual plants represent symbiotic communities with microorganisms associated in or on tissues below- and aboveground.

There are two major classes of fungal symbionts associated with internal plant tissues: fungal endophytes that reside entirely within plants and may be associated with

roots, stems, leaves, or flowers; and mycorrhizal fungi that reside only in roots but extend out into the rhizosphere. In addition, fungal endophytes may be divided into two classes: (1) a relatively small number of fastidious species that are limited to a few monocot hosts (Clay and Schardl, 2002), and (2) a large number of tractable species with broad host ranges, including both monocots and eudicots (Stone et al., 2000). While significant resources and research have been invested in mycorrhizae and class 1 endophytes, comparatively little is known about class 2 endophytes, which may represent the largest group of fungal symbionts. This is partially because the symbiotic functionalities of class 2 endophytes have only recently been elucidated and shown to be responsible for the adaptation of some plants to high-stress environments (Redman et al., 1999b, 2001, 2002b; Arnold et al., 2003; Dingle and McGee, 2003; Ernst et al., 2003).

In this chapter, we focus on symbiotic interactions between class 2 endophytes and a variety of monocot and eudicot host species. Specifically, we will discuss the ability of endophytes to express more than one symbiotic lifestyle, fungal taxonomy vs. lifestyle expression, the adaptive nature of symbioses, mechanisms of symbiotically conferred stress tolerance, and the evolutionary implications of adaptive symbiosis. We will refer to class 2 endophytes as fungal endophytes throughout the text.

34.2 SYMBIOSIS: IT IS ALL ABOUT LIFESTYLES

Historically, fungal symbionts were thought to be restricted to specific symbiotic lifestyles (e.g., mutualism, commensalism, or parasitism; Lewis, 1985). However, recent studies indicate that fungi may express different symbiotic lifestyles in response to host genotypes or environmental factors. For example, depending on the physiological status of plants, some mycorrhizal fungi may be mutualsitic or parasitic (Francis and Read, 1995; Johnson et al., 1997, Graham and Eissenstat, 1998). Moreover, both pathogenic and nonpathogenic fungi are routinely isolated from asymptomatic plant tissues, suggesting that pathogens either express nonpathogenic lifestyles or infect and remain dormant until plant senescence (Schulz et al., 1999). One of the more interesting aspects of lifestyle expression is that the initial phases of infection and colonization by pathogens, mutualists, and commensals are identical for many fungi. Therefore, lifestyle expression is a postcolonization phenomenon and must involve biochemical and or genetic communication between the symbiont and host.

We began investigating the genetic basis of symbiotic lifestyle expression in the cucurbit pathogen *Colletotrichum magna* (Jenkins, 1963; Winstead et al., 1966). Specifically, we were interested in determining if fungal plant pathogens could express nonpathogenic lifestyles. Mutation studies involving UV light or plasmid integration into the nuclear genome revealed that *C. magna* could be converted from a virulent pathogen to a commensal or mutualist by disrupting single genetic loci (Freeman and Rodriguez, 1993; Redman et al., 1999a). The nonpathogenic *C. magna* mutants asymptomatically colonized the roots and stems of cucurbit hosts, and mutualism was defined by the ability to confer resistance against a virulent *C. magna* isolate. This was the first demonstration that the symbiotic lifestyle expressed by one fungal isolate could be changed by mutation and that a pathogen could express nonpathogenic lifestyles. It is not known how many pathogenic fungi can be converted to mutualists or commensals by mutation, but this phenomenon appears to be common among *Colletotrichum* species. We have isolated the nuclear DNA responsible for the conversion of *C. magna* (isolate L2.5) to a mutualist (isolate M68) and constructed a gene disruption plasmid pGM68 (Redman et al., in preparation). When transformed into the wild-type *C. magna* isolate L2.5, the plasmid

integrated by homology (approximately 50%) and generated the same phenotype as expressed by isolate M68. Moreover, when pGM68 was transformed into four other *Colletotrichum* species, the plasmid integrated by homology and generated nonpathogenic mutualistic phenotypes indistinguishable from *C. magna* isolate M68. Although there appear to be few genetic differences between pathogenic and mutualistic lifestyles, lifestyle-altering mutations may be plieotrophic. Moreover, the ability to express pathogenic lifestyles may require fungi to possess the ability to express nonpathogenic lifestyles. It is not yet known if there is a hierarchy to symbiotic lifestyles or how many pathogenic fungi have the ability to express nonpathogenic lifestyles. Regardless, more extensive mutation and host range/lifestyle expression studies are required to address these issues.

Another observation made with the nonpathogenic *C. magna* mutants was that the host range of a fungus is influenced by symbiotic lifestyle expression (Redman et al., 2001). The nonpathogenic mutants of *C. magna* were able to colonize *Colletotrichum*-resistant cucurbit cultivars that the wild-type isolate L2.5 was incapable of colonizing. This prompted a more extensive host range study of the *C. magna* wild type and nonpathogenic mutants involving several plant families (Redman et al., 2001). We found that the wild-type L2.5 and nonpathogenic mutants were able to asymptomatically colonize a variety of plants not previously known to be hosts. Moreover, although the wild-type L2.5 could not colonize as many hosts as the mutant, it could express either parasitic, commensal, or mutualistic lifestyles depending on the host genotype. The nonpathogenic lifestyle expressed by L2.5 was defined as mutualistic based on the ability to confer resistance against virulent pathogens. This was the first demonstration that a fungal pathogen could express a mutualistic lifestyle and confer disease resistance to host plants.

More extensive host range studies with several pathogenic *Colletotrichum* species revealed that fungal pathogens have more flexibility than previously thought in regard to plant host range and symbiotic lifestyle expression (Redman et al., 2001). Our studies indicated that *Colletotrichum* species could be classified into one of three categories:

1. Limited host range capable of only expressing a pathogenic lifestyle
2. Broad host range capable of only expressing a pathogenic lifestyle
3. Broad host range capable of expressing pathogenic, mutualistic, or commensal lifestyles

It is possible that categories 1 and 2 may be a reflection of the limited number of hosts analyzed, and that all of the *Colletotrichum* species have asymptomatic hosts and are able to express nonpathogenic lifestyles.

Historically, the host range of fungal pathogens encompassed plants that exhibited disease symptoms in response to colonization by a particular fungal species. However, it is clear that pathogenic fungi are able to "switch" lifestyles based on the host genotype (Table 34.1). Symbiotic lifestyle switching of fungal pathogens can occur in genetically divergent species (e.g., cucurbit vs. solanaceous species) or in cultivars of the same species (e.g., tomato), suggesting that relatively subtle host differences can alter the communication responsible for the expression of symbiotic lifestyles (Redman et al., 2001; Table 34.2). This is not surprising considering that single gene modifications in fungal pathogens can result in the expression of mutualistic lifestyles. Regardless, the concept that pathogens express a single lifestyle is no longer valid, and the ability to express nonpathogenic lifestyles may explain the presence of pathogenic fungi in the absence of disease so commonly observed in endophyte studies (Schulz et al., 1999).

Table 34.1 Lifestyle Switching of Pathogenic *Colletotrichum* Species in Asymptomatic Hosts

Endophyte	Disease Host[a]	Asymptomtic Host[b]	Symbiotic Lifestyle Switch[c]
C. magna	Cucurbits	Tomato	Mutualist
C. coccodes	Tomato	nf	No switch
C. musae	Banana	Pepper	Mutualist
C. orbiculare	Cucurbits	Tomato	Mutualist
C. lindemuthianum	Dry bean	nf	No switch
C. graminicola	Corn	nf	No switch
C. acutatum	Strawberry	Watermelon	Commensal
C. gloeosporioides	Strawberry	Watermelon	Commensal

[a] Indicates diseased plants from which the isolates were isolated; some species have several disease hosts.
[b] There were several asymptomatic hosts for some species, but only one is listed to indicate lifestyle switching. nf = no asymtomatic hosts found.
[c] Mutualists conferred disease resisitance to asymptomatic hosts, commensals did not confer disease resistance, and no switch indicates that the isolate was pathogenic on all hosts tested.

Data and methods described in Redman et al., *New Phytologist*, 151, 705–716, 2001.

34.3 LIFESTYLE SWITCHING AND FUNGAL TAXONOMY

Symbiotic lifestyle switching adds a new dimension to fungal taxonomy and fungal ecology. It appears that defining fungi based on lifestyle expression may be a tenuous proposition because of host genotype influence. For example, wild-type *Colletotrichum* species may be classified as pathogens, mutualists, or commensals depending on whether they cause disease symptoms or asymptomatically colonize plants and confer disease resistance (Table 34.1). However, when studies are expanded to measure fitness benefits (other than disease resistance) known to be conferred to plants by fungal mutualists, a more complicated pattern of lifestyle expression emerges (Table 34.2). If lifestyle characterization is based on the ability of fungi to confer disease resistance, drought tolerance, or growth enhancement, then *Colletotrichum* species that can switch lifestyles appear to bestow one or more mutualistic benefits. It is too early to tell if all endophytic fungi are capable of switching symbiotic lifestyles and have mutualistic potential. If they do, then it is possible that fungi may have generally evolved as mutualists and that plant disease has become prevalent as a result of transportation of plants around the world. An analysis of the evolution of agriculture on Earth supports this supposition. Between 10,000 and 50,000 years ago, humans began converting from hunting and gathering to crop cultivation. Prior to the era of global conquest and exploration that began more than 2000 years ago, ancient cultures focused on gods thought to be responsible for climate and fertility. During the time of Roman expansion when the spoils of war were returned to what would become Western Europe, a new god, Robigus, the god of crop blight, emerged (Carefoot and Sprott, 1967). Transporting crop plants and seed to new habitats may have introduced the plants to native fungi that were pathogenic on introduced crops, but not on the native plant species. Alternatively, transportation of the new crops and their endophytes into new habitats may have triggered a lifestyle switch. Most likely, both of these scenarios have played themselves out throughout history. Symbiotic lifestyle switching may have started

Table 34.2 Defining the Symbiotic Lifestyle Expressed by Endophytes Based on Host Fitness Benefits

	Tomato, cv Big Beef			Tomato, cv Seattle's Best			Pepper, cv Calif. Wonder		
Endophyte	**Disease Resistance**	**Drought Tolerance**	**Growth Enhancement**	**Disease Resistance**	**Drought Tolerance**	**Growth Enhancement**	**Disease Resistance**	**Drought Tolerance**	**Growth Enhancement**
C. magna	M	M	M	M	M	M	M	M	C
C. orbiculare	M	M	M	P	nd	nd	NH	nd	nd
C. musae	M	C	C	C	nd	nd	M	M	C
C. gloeosporioides	C	M	M	C	C	C	C	M	M

Note: Abbreviations are as follows: M = mutualist, asymptomatic colonization and confers either disease resistance, drought tolerance, or growth enhancement; C = commensal, asymptomatic colonization with no measurable host fitness benefits; P = pathogenic; nd = not determined; NH = nonhost plant that the fungus was unable to colonize.

Data and methods from Redman et al., *New Phytologist*, 151, 705–716, 2001.

a series of famines that ensued in the following centuries. Although this diatribe is based on conjecture, there are many examples in history indicating that a change in plant–fungal interactions occurred that brought about famines considered to be responsible for restricting population growth in Europe for almost 2000 years (Carefoot and Sprott, 1967). Regardless, until the genetic, biochemical, and ecological bases of lifestyle switching and mutualistic benefits are understood, it will be very difficult to characterize fungi based on lifestyle expression.

34.4 MUTUALISTIC FITNESS BENEFITS VS. ENVIRONMENTAL STRESS

It is well documented that mutualistic fungi collectively may confer several host fitness benefits, such as growth enhancement or tolerance to drought, disease, herbivory, and temperature to plants (Malinowski and Belesky, 2000; Redman et al., 2001, 2002b; Clay and Schardl, 2002; Ernst et al., 2003). But it has been unclear if symbioses are adaptive to habitat-specific stresses and, if so, what the temporal requirements for symbiotic adaptation involve. We compared symbioses in two habitats imposing different stresses to determine if symbioses are adaptive. Fungal endophytes were isolated from crop plants in subtropical and tropical habitats and from native plants in geothermal soils of Yellowstone National Park. Based on perceived habitat stresses, the endophtyes were screened for the ability to confer temperature and drought tolerance or disease resistance as described below.

34.4.1 Temperature Tolerance

All plants respond to temperature stress by expressing heat-shock proteins and antioxidant systems, and adjusting osmotic potential and membrane lipids (Iba, 2002). However, few plants thrive in geothermal soils that impose high-temperature stress in the root zone. *Dichanthelium lanuginosum* (panic grass) grows in the geothermal soils that reach temperatures up to 57°C in Yellowstone and Lassen National Parks (Stout et al., 1997; Al-Niemi and Stout, 2002; Stout and Al-Niemi, 2002). These geothermal soils have significant annual temperature fluctuations that are influenced by moisture. Winter snows melt on contact with geothermal soils to decrease temperatures to around 20°C, and a lack of rainfall in summer results in dry, hot soils. Therefore, *D. lanuginosum* is exposed to high root zone temperatures and drought-like conditions on an annual basis.

Two hundred *D. lanuginosum* plants were analyzed and found to be symbiotic with the fungus *Curvularia protuberata* (Redman et al., 2002b). None of the plants analyzed was free of the endophtye, which was isolated from roots, leaves, and seed coats but not seeds. *D. lanuginosum* is the only plant species that occurs in the hot (57°C) geothermal soils and grows as small clusters of individual plants. We surmised that if symbioses were adaptive, then *C. protuberata* may contribute to the thermotolerance and survival of *D. lanuginosum*.

Using a geothermal soil simulator, we observed that the symbiosis between *C. protuberata* and *D. lanuginosum* results in thermotolerance of both symbiont and host plant (Redman et al., 2002b). Nonsymbiotic *D. lanuginosum* has a maximum growth temperature of 40°C, while pure cultures of *C. protuberata* have maximum growth temperatures of 38°C. When these organisms are grown symbiotically, they are able to tolerate daily root temperature regimes of 65°C for 10 h, followed by 37°C for 14 h or sustained 50°C root temperatures (Redman et al., 2002b). After 2 days, symbiotic plants showed no impacts of this temperature regime, while nonsymbiotic plants wilted and died. Five days

after the nonsymbiotic plants died, symbiotic plants still showed no impacts of the temperature regime and the fungal symbiont was isolated from roots. This indicated that both the host plant and fungal symbiont were protected against heat and that the association was mutualistic. The laboratory results prompted a more complicated test under field conditions. Soil was removed from six locations that ranged in temperature from 35 to 45°C, pastuerized to eliminate resident *C. protuberata* spores or mycelia, and placed back into the geothermal sites (Redman et al., 2002b). Clusters of *C. protuberata* colonized (symbiotic) and uncolonized (nonsymbiotic) *D. lanuginosum* plants were transplanted into the pastuerized soils. Twelve months after transplanting, symbiotic plants had greater biomass than nonsymbiotic plants at all temperatures and the biomass differences increased with temperature. At 45°C soil temperatures, the symbiotic plants survived and the nonsymbiotic plants did not (Redman at al., 2002b). This is the first evidence that fungal endophytes can confer thermotolerance and that symbioses with endophtyes may adapt to habitat-specific pressures.

34.4.2 Disease Resistance

Unlike geothermal soil habitats, subtropical and tropical habitats are high in biodiversity. Fungal endophyte surveys indicate that asymptomatic plant tissues are commonly colonized with fungi that are pathogenic in other plant species (Schulz et al., 1999). It is not known if these pathogens are latent or have switched symbiotic lifestyles, but their presence suggests that there is high disease pressure on plant communities in these habitats. One group of pathogenic fungi commonly isolated from asymptomatic plant tissues is *Colletotrichum* species (Pinto et al., 2000; Freeman et al., 2001; Cullen et al., 2002; Manaut et al., 2002). We have demonstrated that several *Colletotrichum* species have the ability to express nonpathogenic lifestyles, including mutualism, depending on the host they colonize (described above). One of the attributes used to classify *Colletotrichum* species as mutualists was the ability to confer disease resistance to asymptomatic hosts (Redman et al., 2001; Table 34.1). In general, disease resistance involved 100% protection against a variety of virulent pathogens. For example, *C. magna* was able to protect plants against pathogenic isolates of *Colletotrichum*, *Fusarium*, and *Phytophthora* (Freeman and Rodriguez, 1993; Redman et al., 2001).

The mechanism of symbiotically conferred disease resistance appears to involve the rapid activation of host defense systems (Redman et al., 1999b). The production of lignin and expression of the enzymes peroxidase and phenylalanine ammonia lyase (PAL) correlate with disease resistance in cucurbit species (Ryals et al., 1996). Therefore, these biochemical activities were monitored before and after symbiotic (colonized with the mutualistic *C. magna* mutant Path-1) and nonsymbiotic watermelon plants were exposed to virulent fungal pathogens. After pathogen challenge, lignin biosynthesis, peroxidase, and PAL activities slowly increased over 4 days in nonsymbiotic plants, and by the fifth day the plants were dead. In symbiotic plants, these biochemical activities increased dramatically within 24 h of pathogen challenge, thereby thwarting ingress of the pathogen. The response in symbiotic plants was so strong that the pathogen could not be reisolated from inoculated tissues (Redman et al., 1999b). This type of disease protection has been defined as endophyte-associated resistance (EAR) and is localized to tissues colonized by endophytes (Redman et al., 1999b).

34.4.3 Drought Tolerance

All plants respond to water deficit through a complex series of biochemical and genetic modifications, including osmotic adjustments, production of antioxidants, altered transcriptional and translational regulation, and altered stomatal activity (Shinozaki and

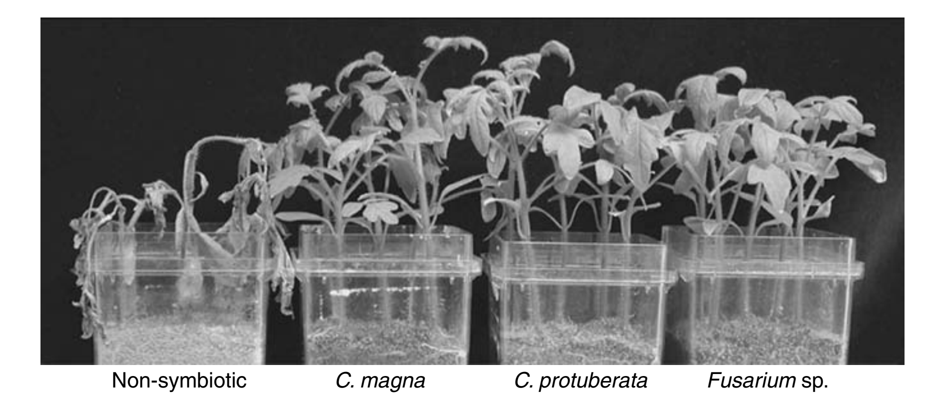


Figure 34.1 **(See color insert following p. 460.)** Nonsymbiotic and symbiotic plants colonized with the indicated endophytes were grown in sand for 2 weeks with adequate watering. Watering was then stopped and plants left to dry. Nonsymbiotic plants wilted after 4 days of desiccation, while the symbiotic plants stayed hydrated for 9 days before wilting.

Yamaguchi-Shinozaki, 1998; Griffiths and Parry, 2002). Yet, few plant species are drought tolerant and survive in habitats with low moisture (Bray, 1997). It is well documented that fungal endophytes confer some level of drought tolerance to plants (Clay and Schardl, 2002). Although the mechanism of endophyte-conferred drought tolerance is unknown, it is thought to involve adjustments in host osmolyte concentrations or stomatal activity (Malinowski and Belesky, 2000). However, endophyte-conferred drought tolerance has been studied in very few plant species. Interestingly, all of the endophytes we have studied, including pathogenic *Colletotrichum* species that can switch lifestyles, confer some level of drought tolerance to plant hosts (Redman et al., 2001; Table 34.2). For example, *Colletotrichum magna* (expressing a nonpathogenic lifestyle) and *C. protuberata* 4666D confer significant drought tolerance to wheat, tomato (Figure 34.1), and watermelon plants. Therefore, drought tolerance appears to be common among fungal endophytes and the communication involved in this mutualistic benefit is conserved between monocots and dicots, which diverged approximately 200 million years ago. This may reflect the fact that when plants moved onto land ca. 400 million years ago, water relations was one of the most difficult stresses to overcome, and fungal symbioses may have developed as a result of symbiotically conferred drought tolerance (Pirozynski and Malloch, 1975).

34.5 HOW ADAPTIVE ARE SYMBIOSES?

We compared the abilities of fungal endophytes from plants thriving in geothermal soils, coastal beaches, or subtropical/tropical habitats to confer fitness benefits to host plants. Endophytes were screened for the ability to confer temperature and drought tolerance, and disease resistance to the plants they were isolated from or on tomato, a host they all asymptomatically colonize (Table 34.3). The endophytes conferred different fitness benefits based on the habitat of isolation. For example, the endophytes from tropical/subtropical habitats, where disease pressure is high, conferred disease resistance, while the endophytes from geothermal soils and coastal beaches did not. Moreover, endophytes from geothermal soils conferred temperature tolerance, while endophytes from the other habitats did not. Endophytes from all three habitats conferred drought tolerance. These

Table 34.3 Mutualistic Benefits vs. Habitat Stress

Endophyte	Habitat	Habitat Stress	Stress Tolerance Conferred by Endophytes[a]		
			Temperature	Disease	Drought
Colletotrichum spp.	Tropical/subtropical agriculture	Disease	0/3	3/3	3/3
Curvularia spp.	Geothermal soils	Heat, dessication	2/2	0/2	2/2
Fusarium and *Alternaria* spp.	Coastal beaches	Dessication	0/3	0/3	3/3

[a] The number on the right of the diagonal indicates the number of endophyte species tested from each habitat, and the number on the left is the number of endophytes conferring the respective stress tolerances.

Methods for these analyses are from Redman et al., *New Phytologist*, 151, 705–716, 2001; Redman et al., *Science*, 298, 1581, 2002b. Data for *Fusarium* and *Alternaria* are unpublished.

results suggest that plant–fungal symbioses adapt to stresses in a habitat-specific manner, a phenomenon we describe as adaptive symbiosis. It is clear that adaptive symbiosis involves communication between endophyte and host that not only regulates symbiotic lifestyle expression, but also regulates symbiotically conferred fitness benefits. This is not surprising considering the complex communication involved in plant symbioses with fungal pathogens, agrobacterium, rhizobium, and mycorrhizae. The temporal requirements for adaptive symbiosis and the communication required for this phenomenon are not known. However, the ramifications of adaptive symbiosis are that specific stress tolerances may be conferred to either monocot or eudicot hosts regardless of the endophyte's origin.

34.6 MECHANISMS OF STRESS TOLERANCE

Symbiotically conferred stress tolerance involves at least two mechanisms: (1) activation of host stress response systems soon after exposure to stress, allowing the plants to avoid or mitigate the impacts of the stress (Redman et al., 1999b; Schulz et al., 1999; Pirttila et al., 2002; Arnold et al., 2003); and (2) biosynthesis of antistress biochemicals by endophytes (Bacon and Hill, 1996; Siegel and Bush, 1997; Strobel et al., 2001; Miller et al., 2002; Schulz et al., 2002). We have proposed that in addition to antistress chemicals, plant–fungal mutualisms have been maintained over evolutionary time because endophytes control the activation of host stress response systems by acting as biological triggers (Rodriguez et al., 2004).

34.7 ECOLOGICAL AND EVOLUTIONARY RAMIFICATIONS

There are four major points presented in the discussion above: (1) individual endophytes can switch symbiotic lifestyles and the outcome of symbiosis is influenced by host genotypes; (2) mutualistic benefits conferred by endophytes are also influenced by host genotypes; (3) the host range of endophytes is poorly defined and may include both monocot and eudicot species; and (4) symbioses adapt to habitat-specific stresses, a phenomenon we describe as adaptive symbiosis. It appears that at least some endophtyes have evolved with a high degree of flexibility to move between genetically distant plant species and communicate in a manner that allows both organisms to survive environmental conditions they cannot survive on their own. This provides endophytes an opportunity to expand habitat range by dispersion of endophyte-colonized plant tissues (rhizomes, seed coats, seed). For example, *C. protuberata* colonizes the seed coats of *D. lanuginosum* found in geothermal soils of Yellowstone National Park. *D. lanuginosum* seeds are borne on panicles that detach from plants and can be carried by the wind over large distances, allowing both the host and symbiont to spread.

By establishing mutualistic symbioses with endophytes, plants may gain new functionalities allowing them to mitigate the impacts of environmental stresses. This could provide a mechanism for plants to make quantum evolutionary changes allowing for habitat expansion and survival in high-stress habitats. However, the dynamics of this process are probably much more complicated than simply forming new mutualisms and must involve the adaptation, physiology, and ecology of fungal endophytes and plant hosts. Ultimately, it is the symbiotic communication between endophyte and host that dictates the outcome of each association and the ability to survive in high-stress habitats.

34.8 SUMMARY

Although endophytic fungi are responsible for the survival of at least some plants in high-stress habitats, more information is required before the full impacts of lifestyle switching and adaptive symbiosis are understood. We do not know yet the limits of adaptive symbiosis with regard to mutualistic benefits that develop in response to habitat-specific stresses. In addition, the temporal requirements for adaptive symbiosis are not known, nor is the fungal ecology associated with endophyte distribution patterns within and between hosts. Although the interspecies communication involved in mutualistic benefits appears to be conserved between monocots and dicots, the biochemical and genetic bases of this communication are not known. Regardless of these knowledge deficiencies, endophytic symbioses offer novel strategies for mitigating the impacts of global change on native plant communities and agricultural crop production.

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35

Biological Soil Crusts and Global Changes: What Does the Future Hold?

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35.1 INTRODUCTION

In arid lands of many diverse climates of the world, where vegetation is sparse or absent, the open ground is not bare but generally covered by a community of small, highly specialized organisms. Together with crustose, foliose, and fruticose, lichenized fungi (i.e., lichens), cyanobacteria, algae, microfungi, and bryophytes aggregate soil particles to form a coherent skin — the biological soil crust (see Chapter 6). Biological soil crusts play an important ecological role worldwide and are a substantial force in shaping the structure and function of many ecosystems. They increase the stability and fertility of soils and influence local hydrological cycles.

Presently, global climate is undergoing changes (Walker et al., 1999) that are likely to significantly modify the abundance, distribution, species composition, and ecological role of biological soil crusts. These changes include substantial increases in atmospheric CO_2 concentrations; increased mean temperatures and changes in extremes and annual temperature fluctuations; changes in precipitation intensity, amount, and timing; altered incident solar radiation with increased ultraviolet input; and interactions between these factors and other variables such as nutrient availability. In addition, land use change and exotic plant invasions are altering habitats on a global scale. Kates et al. (1990) estimates that almost 50% of the ice-free terrestrial land surface has been transformed by humans in ways likely to override any direct effects of changes in atmospheric chemistry (Walker and Steffen, 1997).

In this chapter we will address the likely effects of climate change and changes in land use patterns on biological soil crusts, especially the lichen component. We will examine how these future scenarios might impact the physiological functioning of both individual species and the crust community, as well as expected changes in the species composition of the soil crusts.

35.2 POSSIBLE ECOPHYSIOLOGICAL RESPONSES OF SOIL CRUST LICHENS TO CLIMATIC CHANGES

35.2.1 Increased Level of Atmospheric Carbon Dioxide

Carbon dioxide is the substrate for photosynthesis, and CO_2 response of net photosynthesis (NP) of autotrophs usually follows a saturation type function with an almost linear initial slope. Under conditions of optimal thallus water content (WC), terricolous lichens reach CO_2 saturation in the range between 1000 and 1200 ppm external CO_2 partial pressure (e.g., Nash et al., 1983; Lange et al., 1996, 1999). Lichen species possessing a CO_2 concentrating mechanism tend to have a higher carboxylation efficiency and thereby require lower external CO_2 concentrations for saturation (Palmqvist, 2000). As photosynthesis is not saturated by present ambient natural CO_2 (around 350 ppm), lichens profit from short-term experimental increases in ambient CO_2 with an almost proportional increase in their NP rate if not limited by other factors (e.g., low light or hydration; Figure 35.1). For example, low light under snow cover limits carbon (C) gains even when average CO_2 concentrations are 450 to 500 ppm with peaks to 1641 ppm (Sommerkorn, 2000). Suprasaturation of the lichen thallus by water can impede CO_2 diffusion, drastically increasing the concentrations required for CO_2 saturation (Figure 35.1).

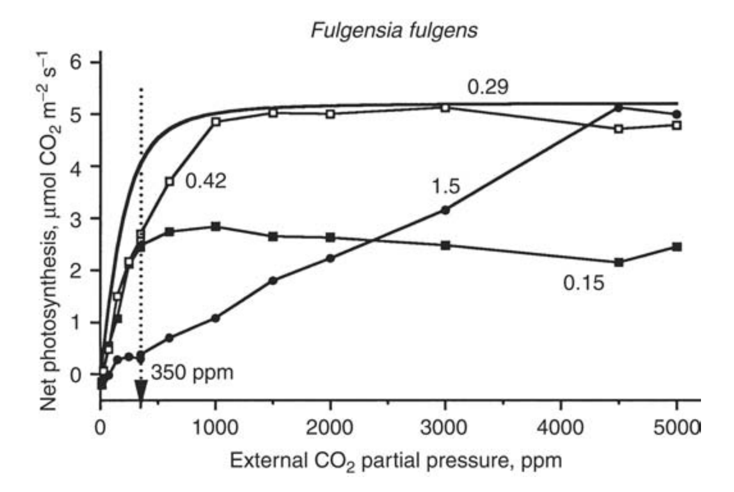


Figure 35.1 Net photosynthesis as a function of external CO_2 concentration at selected water contents (WC, mm precipitation equivalent; see numbers by each curve) of *Fulgensia fulgens* (from soil crust comunity, Hundsheimer Berg, Austria). WC of 0.29 mm denotes optimal hydration. Lower WC (0.15 mm) limits net photosynthesis at about 500 ppm. Suprasaturation changes the initial slope of response curve and increases the CO_2 concentation necessary for saturation (WC of 0.42 mm), which is reached only at 4500 ppm at a WC of 1.5 mm. Natural ambient CO_2 is indicated by dotted line and arrow. (From Lange et al., *Journal of Plant Physiology*, 154, 157–166, 1999. With permission.)

Initial responses of soil lichens to long-term experimental increases in external CO_2 have been inconsistent (reviewed by Tuba et al., 1999). Exposure of *Cladonia convoluta* (a common species in south European soil crusts) for 5 months to 700 ppm CO_2 increased net CO_2 uptake by 50%, a gain that was especially beneficial during drying cycles (Tuba et al., 1998). Tuba et al. concluded that desiccation-tolerant organisms "will be among the main beneficiaries of a high CO_2 future" (p. 39). In contrast, the epiphytic lichen *Parmelia sulcata* (also a common genus in soil crusts) acclimated after only 30 days to 700 ppm CO_2 (Balaguer et al., 1996). When subsequently exposed to 350 ppm CO_2, photosynthetic capacity was reduced, associated with less Rubisco present in the pyrenoid of the algal chloroplasts. The efficiency of photosystem II photochemistry was not significantly changed.

Balaguer et al. (1999) also studied another *Parmelia* species (*P. caperata*) around a natural CO_2 spring, where daytime CO_2 concentrations averaged 729 ppm. When compared with individuals living at 355 ppm CO_2, no evidence of a downregulation was found, in contrast to the above-mentioned laboratory study of the same genus. Carboxylation efficiency, apparent quantum yield, the light-saturated rate of NP, and total thallus Rubisco content were similar among the tested individuals, while the light compensation point of CO_2 assimilation was higher in thalli under elevated CO_2. However, no increase in lichen primary production under elevated CO_2 could be found, although enhanced accumulation of lichen substances was noted. It is not known if the photobiont or mycobiont from the two stands remains genetically identical or if differentiation has taken place under the high CO_2 that has existed for over 200 years.

Because lichen photosynthetic responses to experimentally elevated CO_2 have not been consistent, firm conclusions about the performance of soil crust organisms under future CO_2 conditions are not possible. Response variability may result from other limiting factors (e.g., nutrients) or species-specific differences. Other processes not often measured may be affected, such as nitrogen fixation (Norby and Sigal, 1989). However, it seems unlikely that dramatic CO_2-induced changes in soil crust growth are to be expected, although changes in species composition are possible.

35.2.2 Temperature Changes

Soil crust biota occur within a large gradient of habitats, ranging from hot deserts to cold steppes and polar sites. Often, identical species of cyanobacteria, green algae, and lichens can be found under extremely different temperature regimes. This may be explained by the fact that the photosynthetic carbon assimilation of soil crust lichens is adapted to a broad range of temperatures, from below freezing to temperatures higher than 40°C (see reviews in Kappen, 1988; Nash, 1996). In addition, NP of some species can be almost unaffected by temperatures between 2 and 28°C (*Psora cerebriformis*, Figure 35.2; Lange et al., 1997), or have a broad optimal temperature range (*Diploschistes diacapsis*). In general, cyanobacterial lichens (e.g., *Collema*) are better adapted to high temperatures than chlorolichens, with notable exceptions, e.g., *Squamarina lentigera* (Lange and Green, 2003) and *Acarospora schleicheri* (Nash et al., 1982). Favorable diel net primary production for species such as *S. lentigera* or *Lecanora muralis* depends more on the degree and timing of hydration than temperature, as carbon gains occur during both cold winters and warm summers (Lange, 2003; Lange and Green, 2003). Armstrong (1973) also found a strong correlation between precipitation and monthly growth in Great Britain lichens, with temperature being unimportant. However, Belnap et al. (in press) recently showed *Collema* cover declined dramatically with an increase in June average temperature (r = 0.96).

In addition, the photosynthetic and respiratory processes of lichens can acclimatize to wide seasonal temperature fluctuations, as discovered by Stålfelt (1939) and documented by Kershaw (1985) for arctic and boreal terricolous lichens. *Cladonia convoluta*,

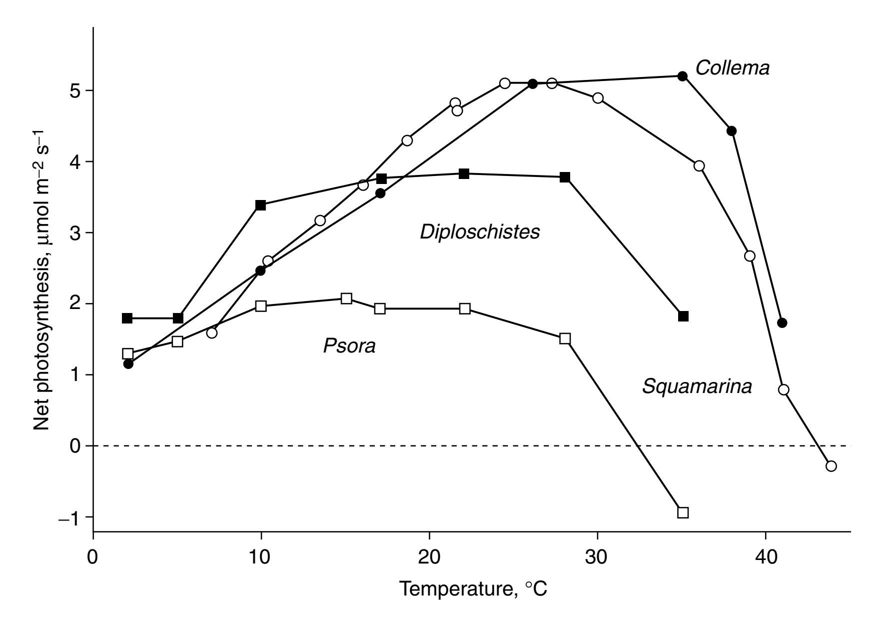


Figure 35.2 Dependence of net photosynthesis on temperature at optimal water content and saturating light for *Psora cerebriformis*, *Diploschistes diacapsis*, *Collema tenax* (from southern Utah, U.S.), and *Squamarina lentigera* (local steppe formation, Würzburg, Germany). (From Lange et al., *Flora*, 192, 1–15, 1997; Lange and Green, *Bibliotecha Lichenologia*, 88, 363–390, 2004.)

Diploschistes muscorum, and *S. lentigera* from a temperate site seasonally alter the temperature sensitivity of dark respiration such that respiratory rates are roughly equal in winter and summer (Lange and Green, 2005). This ensures a greater supply of energy at low temperatures while preventing increased loss of assimilates at higher temperatures.

Because of the low temperature sensitivity of productivity, i.e., the ability to acclimate photosynthetic and respiratory processes under different temperatures, substantial effects of increased temperature on net carbon gain of soil crust communities are unlikely. However, this does not include temperature effects on hydration (see below), nor does it consider effects on other metabolic activities such as nitrogen fixation. Increased temperatures may also stress hot desert lichens in unforeseen ways, such as during their dry-down periods, when they are especially vulnerable to heat stress.

35.2.3 Water Availability

Water availability determines the length and magnitude of metabolic activity time for poikilohydric organisms and, thus, ultimately the productivity and persistence of biological soil crust organisms. It is estimated that soil crusts are metabolically active only 10 to 12% of the year in the Namib Desert (Lange et al., 1991) and photosynthetically active 9 to 11% of the year on the Colorado Plateau, UT (Belnap, unpublished). This proportion might be even smaller for drier regions or those that lack dew or fog. Under such circumstances, even the smallest change in the length of time soils are sufficiently wet for activity will impact the function and perhaps the species composition of the crust community. This was amply demonstrated when changes in crust biota and their photosynthetic productivity were seen at neighboring sites in the Negev highlands that had almost a fivefold variance in maximal dew amounts (Kappen et al., 1980).

Individual soil crust species are also adapted to different types of precipitation. The moisture compensation point of CO_2 exchange for most green algal lichens is very low.

Diploschistes muscorum and *Fulgensia fulgens* represent extreme cases, where precipitation equivalents of 0.04 and 0.055 mm, respectively, are sufficient for activating NP. This equals a dry-weight water content of 12 to 15% and a water potential of about –200 bar, which can be attained via slight dew condensation or vapor from humid air. In contrast, free-living cyanobacteria (e.g., *Microcoleus*) or cyanobacterial lichens (e.g., *Collema*) have a much higher moisture compensation point (Lange et al., 1993, 1998). They require up to five times more water for reactivating NP, which usually occurs only after rainfall. Cyanolichens usually can store much more water than chlorolichens. They can also utilize higher levels of thallus water for photosynthesis before experiencing suprasaturation depression, compared with chlorolichens, which experience suprasaturation depression at relatively low WC (see Figure 6.3). As a consequence, cyanobacteria and cyanobacterial lichens dominate soil crusts in areas where precipitation occurs mainly as rainfall. In contrast, chlorolichens almost exclusively dominate soil crusts in regions where precipitation occurs mostly as dew, fog, or high air humidity. Therefore, changes in the type of precipitation in an area will most likely lead to alteration of the lichen community structure.

Global climate change models predict not only changes in type and amount of precipitation, but also significant changes in precipitation frequency, timing, and interannual variability (Gregory and Mitchell, 1997; Neilson, 1995). Such changes are expected to have profound consequences for soil crust physiological functioning and species composition. Application of smaller, more frequent rainfall events during summer resulted in reduced photosynthetic performance and sunscreen pigment production in *Collema*, compared with lichens receiving larger, less frequent events (Belnap et al., 2004). Summer rain can create repeated short wet–dry cycles, which often result in net C losses and negligible N fixation (Jeffries et al., 1993a, 1993b). These losses may increase mortality or even extirpation of some crust species. This may explain the sharp decreases in lichen and moss diversity observed in hot desert summer rainfall regions in the U.S., Australia, and central Asia relative to winter rainfall areas of equal precipitation. In contrast, increasing summer rainfall in the colder deserts (e.g., northern Great Basin, the Mongolian steppes, or the Arctic) is expected to increase activity time of crust species, as light levels and temperatures are optimal for C and N fixation. Increased biomass, cover, and species richness of the crusts would be expected under this scenario.

35.2.4 Altered Ultraviolet Radiation

Ultraviolet (UV) radiation can be extremely harmful to living organisms, as it interacts directly with their DNA and proteins and affects processes such as photosynthesis, respiration, N_2 fixation, and nutrient uptake (reviewed in Castenholz and Garcia-Pichel, 2000). As radiation penetrates into the soil, light fields become increasingly diffuse. However, irradiance levels are maximal at the soil surface, as both incoming and reflected light combine in this zone. Photobionts in lichens effectively use fungal tissue for protection, with a 90% reduction in UV measured in the center of *Collema coccophorum* (Büdel et al., 1997). Secreted polysaccharides with sunscreen pigments (e.g., scytonemin, mycosporine-like amino acids, melanins, phenolic compounds, and anthraquinones) protect lichens, cyanobacteria, green algae, and microfungi (Garcia-Pichel and Castenholz, 1991). In addition, some cyanobacterial species migrate below the soil surface to avoid radiation exposure, while mosses and lichens can often roll up when dry, possibly to protect sensitive surfaces from radiation damage (Frey and Kürschner, 1991; Rosentreter, 1993).

Recently, progress has been made in analyzing the function of the sun-screening orange-colored anthraquinone parietin in *Xanthoria parietina*, a pigment which protects the photobionts against excessive sun radiation (Gauslaa and Solhaug, 2004). Its synthesis is induced by UV-B (Solhaug et al., 2003), and it shows distinct seasonal acclimation with

low contents in winter and high levels in summer (Gauslaa and McEvoy 2005). The same compound occurs in several typical soil crust lichens (e.g., in *Fulgensia* species) where similar performance might be expected. Although short-term laboratory studies (where organisms are watered) show that soil crust cyanobacteria and lichens respond to experimentally enhanced UV by increasing production of protective pigments, long-term field studies with enhanced UV indicate that crust organisms may often lack the resources needed for such production. In one study, cyanobacteria showed significant mortality after a summer of above-average precipitation, despite large increases in pigment production (Bowker et al., 2002). During summers of average or below-average precipitation, cyanobacteria and *Collema* often experience carbon deficits (Jeffries et al., 1993a, 1993b), and with UV additions, *Collema* showed an additional 57% decline in photosynthetic rates and declines in all measured sunscreen pigments (Belnap et al., 2004). Limited C gain or C losses require the organism to allocate C toward the upkeep of its photosynthetic machinery (e.g., chlorophyll *a*) rather than to the production of UV-protective pigments. This likely results in heightened UV damage and mortality.

As discussed above, increased UV can be expected to impact crust species under many conditions. These effects are expected to differentially affect various crust species, depending on climate factors, the species involved, and the amount and condition of their particular protective mechanisms.

35.2.5 Observed Effects of Climate Change on Soil Crust Lichen Communities

Several theoretical and experimental publications have explored the possible impacts of climate changes on terrestrial lichen performance and distribution (e.g., Bates and Farmer, 1992; Melick and Seppelt, 1994; Nash and Olafsen, 1995; Insarov and Insarova, 1996; Sveinbjörnsson and Sonesson, 1997; Insarov and Schroeter, 2002). Except for catastrophes and small-scale habitat alterations, such changes are likely to be a gradual process, given the low growth and slow successional rates of lichens. Lichen floras in Europe were impoverished by atmospheric SO_2 pollution. Studies since 1980 show a slow but continuous recovery (Nimis et al., 2002). Such slow response makes the study of climate change effects on lichens difficult.

Nevertheless, some changes in lichen species composition are already becoming apparent. A study of many sites in the Netherlands clearly demonstrates that recently increased temperature and precipitation have already resulted in floristic changes in the surveyed lichen communities (van Herk et al., 2002; see also Aptroot and van Herk, 2001). Changes over the last 22 years in the distribution of 329 epiphytic and terricolous lichens were correlated with the latitudinal distribution and ecological determinates of the different species. According to van Herk et al. (2002) and Aptroot (personal communication), in the Netherlands soil lichen species with predominantly boreal distribution are showing a decline while subtropical species are increasing. Changes in species between 1995 and 2001 appear to be positively correlated with both temperature and nutrient demand, with a recent and significant shift toward species preferring warm circumstances, independent from and concurrent with changes due to nutrient availability (van Herk et al., 2002). Soil lichen species with predominantly boreal distributions (e.g., *Cetraria islandica*, *Cladina rangiferina*) and those preferring somewhat cooler conditions (*Dibaeis baeomyces* and *Pycnothelia papillaria*) are declining or have totally disappeared from relatively undisturbed heathlands (Aptroot, personal communication). In contrast, soil crust lichens with a Mediterranean center of distribution (e.g., *Fulgensia fulgens* and *Endocarpon pusillum*) have not changed their distribution. *Placynthiella oligotropha*, one of the terricolous

species expanding the most successfully, is a warm-temperate lichen. From these impressive analyses, it seems clear that global changes have already begun to impact the distribution of lichens, including those found in soil crust communities.

Soil crust organisms are generally unable to compete with phanerogamous plants for light and space, and they colonize the ground in the gaps of vegetation where climate conditions prevent closed plant canopies. Therefore, secondary effects of climate changes on soil crust distribution and composition due to changes in the vascular plant community also need consideration. Large increases in net primary productivity may occur in arid ecosystems due to elevated CO_2 and enhanced water availability (Melillo et al., 1993). Early results from a Mojave Desert elevated CO_2 site report an increase in cover and biomass of the invasive annual *Bromus tectorum* (Smith et al., 2000). Increased annual grass will shade soil crusts, decrease space available for colonization, and increase fire cycles. Sites dominated by *Bromus* are 10 to 500 times more likely to burn than uninvaded sites (Knapp, 1996), and fire intervals can decrease from >100 years to <5 years (Whisenant, 1990). Fire kills soil crust organisms; thus, increased fire frequency reduces crust biomass and precludes colonization of lichens and mosses. *Bromus* also increases seed availability, which then attracts burrowing rodents. Attendant soil disturbance prevents development of perennial lichens and mosses, and soil crusts are instead dominated by cyanobacteria and annual mosses. Such changes in soil crust species composition result in decreased species richness and rates of N and C inputs into soils.

Cornelissen et al. (2001) studied the interaction between phanerogamous plants and terricolous macrolichens in different arctic tundra and heath ecosystems containing fruticose and foliose lichens such as *Cladina* spp., *Cetraria nivalis*, *Stereocaulon alpinum*, and *Thamnolia* sp. The authors hypothesized that climate warming and increased nutrient availability in the more climatically mild arctic ecosystems, with relatively dense canopies, would result in a decline in macrolichen abundance as a function of increased vascular plant abundance. In contrast, they expected such a relationship to be absent in the more open high-arctic or arctic-alpine plant communities. This hypothesis was clearly supported by data from ecosystem manipulation experiments and with comparisons along natural environmental gradients.

One consequence of global climate change will unquestionably favor development of biological soil crusts. Increasing temperature and the subsequent retreat of polar and high montane glaciers (Oechel et al., 1997) will create additional substrates for the colonization of soil crust organisms. As a pioneering vegetation type, these crusts are important for stabilizing soils and increasing their fertility (Hansen, 2003; Türk and Gärtner, 2003).

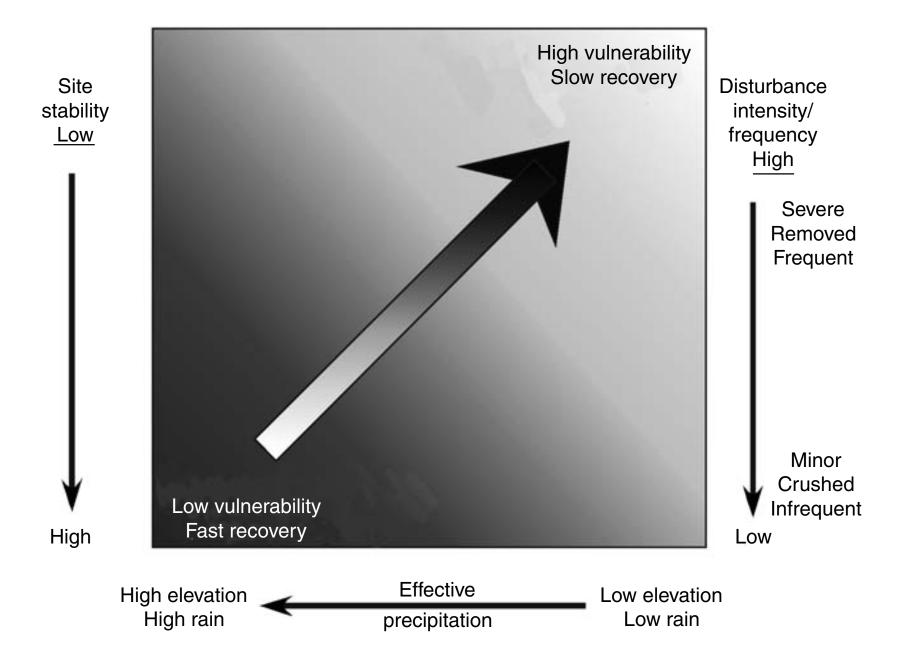
Evidence is accumulating that the global climate changes observed in the past few decades are directly and indirectly impacting biological soil crusts, including their extent, distribution, and species composition. However, it is certainly still too early to predict the direction of change or the details of these changes.

35.3 LAND USE AND LICHENS IN SOIL CRUST COMMUNITIES

While changes in land use are of global concern, increasing use of marginal lands is especially widespread in the arid and semiarid regions where biological soil crusts are most apparent. These regions are home to 35% of the world's population (Brooks and Pokshishevsky, 1986) and conversion of these lands for agricultural and recreational use is rapidly increasing (Brown et al., 1995).

35.3.1 Surface Disturbance

Compressional and shear forces are generated by both agricultural (trampling by livestock) and recreational (trampling by people, crushing by vehicles) uses. Biological soil crusts are highly vulnerable to this type of disturbance, especially when dry and therefore brittle, with lichens the most susceptible crust component. The vulnerability of a specific soil crust to disturbance is heavily influenced by soil texture, soil moisture, and the type and intensity of disturbance (Figure 35.3). Soil crust organisms on coarse-textured soils are



Factors determining site stability

	Low stability	High stability
Soil texture/age	Coarse/young	Fine/old
Rock/gravel cover	Rolling	Embedded
Sand deposition	High	Low
Plant spacing	Sparse	Dense
Slope	Steep	Flat

Figure 35.3 Vulnerability and recoverability of crusts depend on gradients of site stability, effective precipitation, and disturbance regimes. Top panel: Crusts at sites with the greatest stability (defined in bottom panel), greatest effective precipitation, and lowest disturbance frequency or intensity will be less impacted (dark shading) than crusts at sites with lower stability, less effective precipitation, and higher disturbance frequency or intensity (light shading). Similarly, crust recovery time is faster (dark shading) in areas of low vulnerability and slower (light shading) where vulnerability is higher. Bottom panel: Factors influencing site stability. (Adapted from Belnap and Eldridge, in *Biological Soil Crusts: Structure, Function, and Management*, J. Belnap and O.L. Lange, Eds., Springer-Verlag, Berlin, 2003, pp. 363–383.)

more vulnerable than silty and clay soils when dry, whereas clay soils are the most vulnerable when they are wet. Crusts on gravel-covered soils are the least vulnerable. Disturbance that churns the soils and buries organisms (e.g., accelerating vehicles) is much more destructive to crusts than disturbance that crushes crusts in place (e.g., slow walking). Repeated disturbance also buries crust material and, thus, is more damaging than occasional use. Lichens are the most susceptible to disturbance, followed by mosses, smaller cyanobacteria and green algae, and lastly, the large cyanobacteria.

Indirect effects of soil surface disturbance occur when destabilized soils bury or "sandblast" adjacent biological crusts, resulting in the death of the photosynthetic components of the soil crust. Direct effects occur when the brittle crust organisms are crushed and then buried, washed, or blown away (Harper and Marble, 1988; Campbell et al., 1989; Belnap, 1993). Soil crusts in undisturbed areas can have up to 20 to 30 species of soil lichens and mosses and up to 60 species of cyanobacteria, whereas adjacent disturbed areas often have no lichens or mosses and only a few species of cyanobacteria.

Resistance to wind and water erosion has been shown to parallel biological crust development, as bare soils or cyanobacterial crusts allow up to 35 times more wind and water erosion than lichen-moss crusts (McKenna-Neuman et al., 1996; Belnap and Gillette, 1997, 1998). Disturbed soil crusts are often only a few millimeters thick, in contrast to undisturbed crust that can be up to 10 cm thick (Belnap, 1995). Therefore, disturbance that results in cyanobacterial crusts leaves soils more vulnerable to erosion than those dominated by lichen crusts.

In cool regions, the cohesive soil crust is frost-heaved upward in winter and then differentially eroded downward, creating a greatly roughened soil surface. This roughened surface decreases the velocity and erosivity of wind and water. Disturbance by livestock, people, or vehicles flattens these soil surfaces, increasing erosion susceptibility. Flattening reduces residence time of the water, decreasing water infiltration. Loss of lichens and mosses also reduces the water storage capacity of the soil. Therefore, such soil surface disturbances can greatly accelerate soil loss and reduce soil moisture in these regions. In contrast, soil crusts in hyperarid regions where soils do not freeze actually smooth the soil surface. In these regions, disturbance roughens the soil surface. This increases soil erosion and increases water infiltration (reviewed in Belnap and Eldridge, 2003). However, localized infiltration in hyperarid regions is not always desirable, as it can result in the death of downslope plants (Tongway and Ludwig, 1990). Erosion is not desirable in any desert, as soils take 5,000 to 10,000 years to form in these areas (Dregne, 1983).

Disturbance to the soil crusts reduces soil fertility and moisture retention. Lichens and mosses fix more C than the equivalent surface area of cyanobacteria, and therefore their loss reduces soil C inputs. Reduction of total crust biomass also means less secretion of growth factors, chelators, and acids to free carbonate-bound phosphorus. Nitrogen fixation declines 60 to 100% immediately following disturbance (Belnap, 2003), and the subsequent death of buried N-fixing cyanobacteria and lichens results in virtual elimination of new N inputs into soils. This, coupled with continued N losses from gaseous emissions and erosion, causes decreases in soil and plant N (Ehleringer et al., 1998; Evans and Belnap, 1999). In addition, the loss of soil fine particles to which nutrients are attached directly reduces soil fertility and water-holding capacity of desert soils. Soil surface disturbance also favors invasion of annual grasses. The presence of invasive grasses leads to the replacement of lichens by mosses and cyanobacteria, with a subsequent decline in C and N inputs to soils (for a more complete discussion on invasive grasses, see Section 35.2.5).

The conversion of lichen-moss crusts to cyanobacterial crusts, with concomitant declines in C and N inputs, decreases the abundance and diversity of soil food webs and

thereby affects nutrient cycling rates and nutrient availability (Belnap, 2003b). Nitrogen mineralization can decrease ~80% following the loss of lichens and mosses. Disruption of soil food webs can reverberate throughout the ecosystem, affecting vascular plants and faunal components (Coleman et al., 1992; Hendrix et al., 1992). Preventing desertification depends on maintaining stability and fertility of soils and the diversity of processes and species in ecosystems (Dregne, 1983); thus, loss of lichen-moss crusts can accelerate the desertification process.

Loss of lichens and mosses also impacts other ecosystem characteristics. Lichen-moss crusts have 50% less reflectance of wavelengths from 0.25 to 2.5 μm than bare soil (Belnap, 1995). This represents a change in the surface energy flux of approximately 40 W/m^2 and temperature differences of up to 14°C (Belnap, 1995). Soil temperatures affect many physiological process rates, including N and C fixation, microbial activity, plant nutrient uptake, and timing of seed germination. Food and other resources are often partitioned among invertebrates and small mammals on the basis of surface temperatures (Doyen and Tschinkel, 1974; Wallwork, 1982; Crawford, 1991). Many small desert animals are weak burrowers, and soil surface microclimates are of great importance to their survival (Larmuth, 1978). Consequently, altering surface temperatures can affect many desert organisms.

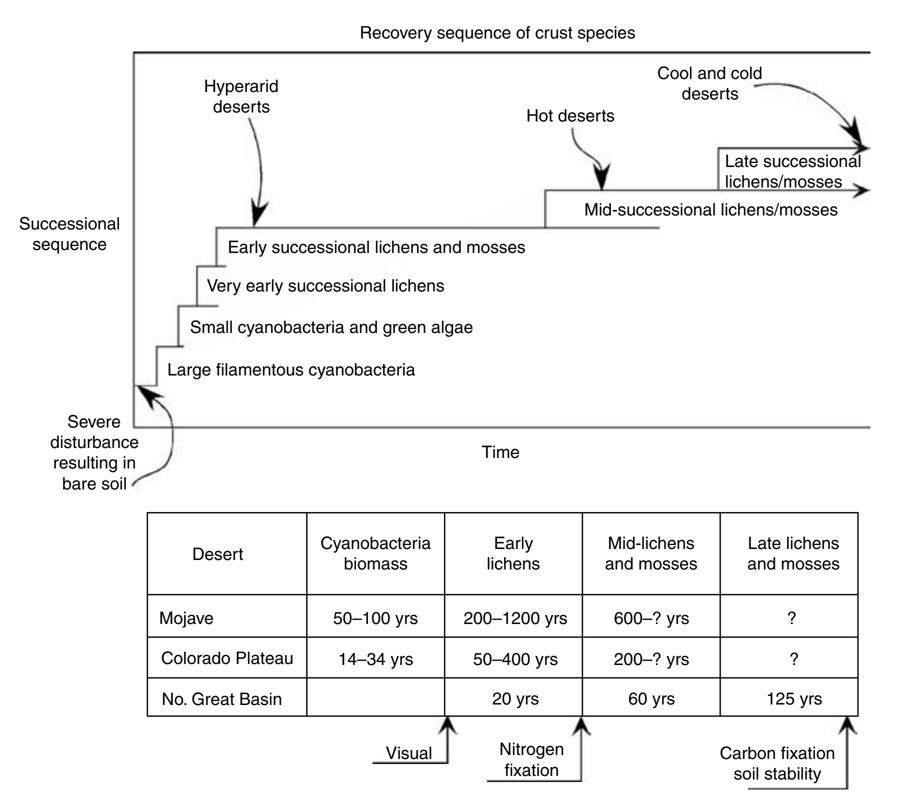
Disturbance of biological soil crusts has many detrimental effects on desert ecosystems. Conversion of lichen-moss soil crusts to cyanobacterial soil crusts means loss of fertility and stability for desert soils. These changes lead to impacts on many other aspects of desert ecosystems.

35.3.2 Recovery

Estimates of recovery times for biological soil crusts after disturbance vary widely in the literature, partially due to different assessment techniques and the lumping of different climates and crust types. Accurate assessment of recovery rates is difficult, as they depend on many factors, with the most important being soil stability and fertility; the type, intensity, and extent of disturbance; the availability of inoculation material; the predisturbance flora; and the temperature and moisture regimes that follow disturbance events (Figure 35.4; reviewed in Belnap and Eldridge, 2003). Coarse soils, with their inherent instability, low fertility, and low water-holding capacity are slower to recover than fine-textured soils with high water-holding capacity and greater fertility.

Sites receiving severe disturbance that removes crust material are slower to recover than sites receiving less intense disturbance that crushes organisms in place, leaving them to act as inoculating material. Disturbances with large surface-to-volume ratios have a slow recovery, as most colonization occurs from adjacent, undisturbed areas. Cyanolichens generally recover faster than chlorolichens, perhaps due to cyanobacterial photobionts being much more common in desert soils than green algal photobionts, thus facilitating colonization by cyanolichen spores. Because crust organisms are metabolically active only when wet, regions with low potential evapotranspiration recover much more quickly than crusts in regions with high potential evapotranspiration (Belnap and Eldridge, 2003).

In severely disturbed areas, large filamentous cyanobacteria (e.g., *Microcoleus*) generally colonize first, followed by smaller cyanobacteria, green algae, and microfungi (Figure 35.4). After soils are stabilized, early successional lichens (e.g., *Collema*) and mosses colonize. Where there is sufficient precipitation (e.g., cool deserts), these species are followed by later successional species (e.g., *Diploschistes*). Recovery of the ecological functions (e.g., C and N fixation) of the soil crusts depends on what species recolonize. Often, mosses may colonize areas previously dominated by N-fixing lichens. Consequently, N in soils and plants may take much longer to recover than expected (Evans and



Desert	Cyanobacteria biomass	Early lichens	Mid-lichens and mosses	Late lichens and mosses
Mojave	50–100 yrs	200–1200 yrs	600–? yrs	?
Colorado Plateau	14–34 yrs	50–400 yrs	200–? yrs	?
No. Great Basin		20 yrs	60 yrs	125 yrs

Figure 35.4 Colonization sequence and estimated recovery times for crustal species in the western U.S. Top panel: Arrows indicate the degree of crust development possible in a desert type; length of line indicates relative time for recovery of each successional group. Species indicative of successional groups include large filamentous cyanobacteria, *Microcoleus* spp.; small cyanobacteria, *Nostoc* spp.; very early successional, *Collema* spp.; early successional, *Placidium* spp., *Pterygoneurum* spp.; mid-successional, *Psora* spp., *Fulgensia* spp., *Tortula* spp., *Bryum* spp.; late successional, *Acarospora* spp., *Pannaria* spp. Bottom panel: Relative recovery rates for different climates. Reported estimates are averages, as sites show considerable variation in recovery times for sandy soils and are based on linear extrapolations. Recovery rates of mid- and late-successional species are not known in drier deserts, where slow recovery times have precluded estimates. Estimates are based on published rates. (Adapted from Belnap and Eldridge, in *Biological Soil Crusts: Structure, Function, and Management*, J. Belnap and O.L. Lange, Eds., Springer-Verlag, Berlin, 2003, pp. 363–383.)

Belnap, 1999). Restoration of normal surface albedos, C fixation, and soil stability require all predisturbance species to recolonize, especially lichens and mosses.

Inoculants can be used to speed up recovery of soil crusts (Tiedemann et al., 1980; Ashley and Rushforth, 1984; St. Clair et al., 1986; Belnap, 1993; Buttars et al., 1998). Cyanobacterial inoculants are being developed, but as of yet are unsuccessful for large areas. The difficulty of growing lichens and mosses in the lab will likely preclude inoculant development. The current lack of commercially available products requires that intact crusts be used as the inoculating material, limiting this technique to use in small areas.

Unfortunately, many activities associated with humans are incompatible with the well-being of soil crusts. These organisms are easily crushed, and once lost, recovery is often slow, especially for the mid- and late-successional lichen component. Therefore, reducing disturbance is the best management strategy.

35.4 CONCLUSIONS

A recent review by Hughes (2000) showed changes in the physiology, distribution, and phenology of trees, grasses, forbs, phytoplankton, butterflies, mosquitoes, oceanic and terrestrial birds, reptiles, amphibians, and insects in response to global warming. Recently, Aptroot and van Herk (2002, p. 57) concluded: "It can be safely predicted that global warming would have an influence on lichen floras." Reviews by Insarov and Insarova (1996) and Insarov and Schroeter (2002) that focused on how climate changes will affect lichens, including terricolous species, came to a similar conclusion.

Autotrophic poikilohydric organisms, such as those found in biological soil crusts, are metabolically active only when wet. Therefore, they are highly responsive to the slightest changes in water availability (both amount and timing). Temperature influences metabolic processes such as photosynthesis and nitrogen fixation, while also determining rates of water loss and thus duration of metabolic activity. Any change in these parameters will impact soil crust structure and function, as can be seen by correlating current species distributions with environmental conditions. Additionally, increases in CO_2 and UV are expected to have substantial impacts on soil crusts.

Increasing use of arid and semiarid lands, with attendant soil surface disturbance and invasion of exotic plants, will most definitely produce profound changes in soil crusts. The soil crusts are fragile systems highly vulnerable to such disturbance. Heavy livestock grazing, human trampling, and off-road vehicles crush the brittle crust, destroying its structure and changing its species composition, thus reducing soil stability and productivity. As the species composition and physiological functioning of crust components are changed through these different forces, we can expect concomitant changes in soil food web structure and function, nutrient cycling rates, vascular plants, and fauna. As these disturbances increase, crust conservation needs to become an even more important issue for land managers.

Because the photosynthetic response of lichens to elevated CO_2 has not been consistent and because information about long-term exposure is scarce, firm conclusions about the performance of soil crust organisms under future CO_2 conditions are not yet possible. Observed limitations and the variability of responses may result from factors other than elevated CO_2 (e.g., nutrients and light). The situation might be similar for mature forest trees, where little (if any) effects via CO_2 fertilization on growth are to be expected (Körner, 2003). Other lichen processes not often measured may also be affected, such as nitrogen fixation (Norby and Sigal, 1989). However, it seems unlikely that dramatic CO_2-induced changes in soil crust growth will occur. Changes in species composition might be possible, due to species-specific differences in the presence or activity of carbon-concentrating mechanisms, which will determine the CO_2 sensitivity of photobionts' photosynthesis to atmospheric CO_2.

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36

Nutrient Acquisition Strategies of Fungi and Their Relation to Elevated Atmospheric CO_2

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36.1 INTRODUCTION

Effects of elevated atmospheric CO_2 on ecosystems have been fairly well studied within the past few decades. At this point, certain general responses have become apparent, including an approximate 30% augmentation of plant productivity (Poorter, 1993), increases in root:shoot ratio (Bazzaz, 1990; Rogers et al., 1994), reduction of stomatal conductance (Jackson et al., 1994; Field et al., 1995; Medlyn et al., 2001), and shifts in plant communities (Ehleringer et al., 1997). This knowledge enables us to improve our predictions of ecosystem function under future environmental conditions expected within this century. Moreover, by exposing organisms to perturbations in the environment and examining how they respond, we can learn much about their basic ecology. For example, we can delineate functional groups and niches, and examine species interactions under different conditions. Therefore, global change research has implications for both applied and basic science, and our understanding of plant ecology has improved considerably as a result of this focus.

The same consideration applies to microbial ecology, and researchers are now well poised to apply recent molecular and technological advances to intensive investigations of microbial communities under elevated CO_2. Traditionally, ecosystem studies have treated microbes as a black box and have measured responses of the community as a whole. Soil respiration and microbial respiration usually increase when CO_2 concentrations are doubled, but the degree of response is highly variable and often nonsignificant (Zak

et al., 2000b). Microbial biomass shows no particular trend (Zak et al., 2000b). Within the microbial pool, functional groups may respond differently to diverse effects of elevated CO_2 on the soil environment, and these differences may contribute to variation among studies. In fact, community composition of microbes is frequently altered (e.g., Klironomos et al., 1996; Grayston et al., 1998; Jones et al., 1998; Hungate et al., 2000; Olszyk et al., 2001; Phillips et al., 2002). By examining responses of individual groups to elevated CO_2, we may determine the causes underlying large-scale effects, as well as improve our understanding of microbial ecophysiology and community dynamics. In this chapter, I will discuss the potential mechanisms by which elevated CO_2 could influence the soil community; I will apply economic-based principles of resource allocation to predict how traits of microbial species should relate to elevated CO_2; and I will examine evidence for corresponding shifts in microbial community composition. Particular attention will be paid to mycorrhizal and saprotrophic fungi.

36.2 POTENTIAL MECHANISMS FOR CO_2 EFFECTS

Fungi have been shown to respond directly to CO_2 concentrations, but only when those concentrations are on the order of 3.3 to 15% or higher (but see Jensen, 1967; Lockhart, 1967; Macauley and Griffin, 1969; Highley et al., 1983; Conway et al., 2000). Concentrations of CO_2 in the soil are usually between 0.3 and 1.5%, well below that range (Sadowsky and Schortemeyer, 1997). By comparison, the projected increase in atmospheric CO_2 concentrations from 380 to ~700 ppm (i.e., 0.038 to 0.070%) within the century is small, and fungi are unlikely to be directly affected (Rillig et al., 2002). However, plants are sensitive to these atmospheric changes and may be particularly important in mediating microbial effects.

Perhaps the most widely studied CO_2 effect on fungi is the response of mycorrhizal groups. Because these symbionts receive carbohydrates directly from plants, they should be directly influenced by the increased photosynthetic capacity of their hosts under elevated CO_2. Nitrogen (N) or phosphorus (P) tend to become more limiting to plant growth under these conditions (Oren et al., 2001; Schlesinger and Lichter, 2001; Finzi et al., 2002), so plants may allocate excess carbon (C) to mycorrhizal fungi in order to improve nutrient acquisition (Read, 1991). Both arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi often become more abundant under CO_2 enrichment, whether measured as total root colonization (Diaz, 1996; Hodge, 1996; Staddon and Fitter, 1998; Cairney and Meharg, 1999) or as hyphal length in the soil (Treseder and Allen, 2000).

Unlike mycorrhizal fungi, saprotrophic fungi rely on C derived from plant litter or exudates. Both sources can be altered by elevated CO_2. As net primary productivity tends to increase (Bazzaz, 1990; Rogers et al., 1994), inputs of leaf and root litter to the soil increase as well. In addition, exudation of low-molecular-weight C compounds from roots can rise (Norby et al., 1987; Lekkerkerk et al., 1990; Zak et al., 1993; Griffiths et al., 1998), although occasionally no CO_2 effect is observed (Whipps, 1985; Rouhier et al., 1994). Altogether, a larger substrate pool should be available for metabolism by saprotrophic fungi. This trend may be responsible for commonly observed increases in abundance of saprotrophic fungi (Dhillion et al., 1996; Klironomos et al., 1997b; Grayston et al., 1998; Hungate et al., 2000; Olszyk et al., 2001; Phillips et al., 2002), although occasionally there is no change (Kampichler et al., 1998; Insam et al., 1999) or a decline (Klironomos et al., 1997a).

Modifications in the quality of plant litter under CO_2 enrichment are particularly likely to alter microbial community structure. Specifically, a recent meta-analysis of CO_2

studies revealed that lignin concentrations in leaf litter increase significantly, while N concentrations decline (Norby et al., 2001). Roots have been analyzed less often, but where they have been measured, C:N ratios increase (Jackson and Reynolds, 1996; Berntson and Bazzaz, 1997, 1998; Franck et al., 1997; Pregitzer et al., 2000; Van Kessel et al., 2000) more often than not (Crookshanks et al., 1998; Kampichler et al., 1998; Matamala and Schlesinger, 2000). Condensed tannins and phenolics can also become more prevalent (Gebauer et al., 1998; Zak et al., 2000b). Overall, litter quality generally declines in plants under elevated CO_2, although decomposition rates are not necessarily affected (Norby et al., 2001).

This shift in litter chemistry has potential consequences for shifts in communities among — and within — major groups of microbes. For example, reduced litter quality could favor fungi over bacteria because fungi generally have a greater capacity for metabolism of recalcitrant compounds than do bacteria (Paul and Clark, 1996). Researchers have observed that ratios of fungal:bacterial biomass increase (Klironomos et al., 1996; Grayston et al., 1998; Jones et al., 1998; Hungate et al., 2000; Olszyk et al., 2001; Wiemken et al., 2001; Phillips et al., 2002) more often than not (Zak et al., 1996, 2000a; Insam et al., 1999) under elevated CO_2. Within fungi, shifts between saprotrophic and mycorrhizal groups are possible and should be influenced by a number of factors, including nutrient limitation of plants, which could determine the extent to which allocation of C to mycorrhizal fungi is augmented; and nutrient limitation of saprotrophic fungi, which could influence the ability of that group to exploit additional C substrates in litter. Empirical studies indicate that elevated CO_2 can enhance mycorrhizal:saprotrophic ratios when soil fertility is relatively low, but not necessarily at higher soil fertility (Klironomos et al., 1996, 1997a). Responses also vary among plant hosts (Rillig et al., 1998). At the species level, because fungi can specialize on the metabolism of particular chemical compounds (e.g., Abuzinadah and Read, 1986; Hodge et al., 1995; Anderson et al., 1999; Cairney, 1999; Yamanaka, 1999), different saprotrophic fungal species may dominate under elevated CO_2, depending on specific changes in plant chemistry.

Plants could serve as mechanisms for CO_2 effects in other ways. For example, plants might become stronger competitors for soil nutrients due to increased root growth, and in this way curtail growth of saprotrophic or mycorrhizal fungi. In addition, because stomatal conductance frequently declines under elevated CO_2, transpiration is often reduced, with a resulting increase in soil moisture. Microbes can be particularly sensitive to water availability due to high surface-to-volume ratios, and fungal species could vary in drought tolerance.

In a study based in terracosms of Douglas fir, Lin et al. (1999) found that the oxidation of soil organic matter decreased 14% under elevated CO_2, but litter decomposition increased 18%. Since these measurements were made early in the study when litter quality had not yet changed substantially, other modifications in the soil environment, such as plant–microbe competition or water availability, may have been responsible. Alterations in the structure of the microbial community could also have contributed. However, it is difficult to separate microbial community effects from other environmental effects when studying decomposition, and this approach has rarely been used within the context of CO_2 research (Norby et al., 2001). One way to examine this issue is to focus on the ecological traits of abundant or keystone microbes. We can hypothesize which functional groups should predominate under elevated CO_2 vs. ambient CO_2, and then verify shifts in community composition in empirical studies. Using this information, and by relating specific groups to their functions in ecosystems, we can begin to clarify effects of community composition on large-scale processes, such as decomposition, soil C storage, and soil respiration.

36.3 NUTRIENT ACQUISITION STRATEGIES OF FUNGAL GROUPS

Elevated CO_2 augments the input of C into ecosystems via plants, and in doing so may either ameliorate C limitation or accentuate N or P limitation of microbial growth. Analogous alterations in nutrient limitation have been observed for plants (Oren et al., 2001; Schlesinger and Lichter, 2001; Finzi et al., 2002). This shift in the balance of nutrients may elicit a shift in the balance of microbial groups that vary in demands for C, N, or P. Specifically, fungal groups that are tolerant of low ratios of C:N or C:P availability might become rarer under elevated CO_2 because they could be outcompeted by fungi that require more C and less N or P. In discussing this issue, it becomes useful to divide fungi into nutrient-related functional groups: low C, low N, and low P. There may be some degree of phenotypic plasticity within species. Nevertheless, given that physiological and energetic trade-offs are likely to exist between the different strategies, most fungal species might be strong competitors under low C, low N, or low P conditions, but not all. I hypothesize that communities shift from low C fungi to low N or P fungi under elevated CO_2, due to increases in C:N or C:P availability.

What ecological characteristics should be typical of these functional groups of fungi? Two decades ago, Bloom et al. (1985) addressed a similar question for plants by applying economic theories of resource limitation. We can use this approach for fungi as well because many of the same major principles are relevant. For example, a fundamental assumption is that organisms (analogous to business firms) require multiple types of resources, such as C, N, or P. However, these resources are not necessarily available in the environment in the same proportions required by the organism. Often, one or more resources are limiting to growth, while others are available in excess. In order to improve growth rates, excess resources should be invested in the acquisition of limiting resources. Growth rates are optimized if all resources are equally limiting to growth, although in practice this condition is rarely obtained. In relation to fungi, individuals that are C limited should invest other nutrients in C acquisition, while those that are N or P limited should invest C in soil nutrient acquisition. These strategies would be employed by the low C and low N/P functional groups, respectively.

Many of the ideas that have been developed regarding nutrient acquisition strategies of plants (Chapin, 1980; Bloom et al., 1985; Martinelli et al., 1999; Aerts and Chapin, 2000; Treseder and Vitousek, 2001) are relevant to mycorrhizal fungi in particular (Table 36.1). For both taxa, C sources are spatially segregated from N and P sources. In plants, resources can be allocated either aboveground for photosynthesis and C capture, or belowground for N and P foraging. Similarly, mycorrhizal fungi acquire C by colonizing plant roots, but N and P are taken up from the soil. Because resources available for tissue construction are finite, allocation must be divided between shoots and roots for plants and between intra- and extraradical structures for AM fungi (Johnson et al., 2003). According to economic theory, I expect that low C mycorrhizal fungi will possess a greater proportion of biomass in arbuscules (AM fungi) or ectomycorrhizal root sheaths (ECM fungi) than would low N/P species. Excess N and P would essentially be traded for C acquisition via these structures. Another consideration is that AM fungi can become N or P limited in soils with low fertility (Treseder and Allen, 2002), and low N or P species would be expected to invest more C in the production of external hyphae in order to better exploit the soil for nutrient scavenging (Johnson et al., 2003). Moreover, species particularly adapted to low P conditions may exhibit finer hyphal branching than do those adapted to low N conditions because phosphate is less mobile in the soil than are ammonium and nitrate. Runner hyphae (AM) or rhizomorphs (ECM) would be more prevalent in low N/P fungi, as they enable

Table 36.1 Functional Traits of Mycorrhizal Fungi Expected under Nutrient Limitation

Function	AM Trait	ECM Trait	Analogous Plant Trait	C Limitation	N Limitation	P Limitation
Exchange N and P for C	Arbuscules or hyphal coils	Root sheaths	Photosynthetic capacity	+	-	–
N and P uptake from soil	External hyphae	External hyphae	Roots, mycorrhizae	–	+	+
Local foraging for P	Fine hyphal branches	Fine hyphal branches	Fine root branching	0	0	+
Long-distance foraging for N and P	Runner hyphae	Rhizomorphs	Coarse roots	–	+	+
C storage (lipids)	Vesicles		Starch storage	+	0	0
P storage	Polyphosphate accumulation in hyphae	Phosphorus accumulation in root sheaths	Luxury consumption of phosphorus	0	0	+
Phosphorus mineralization	Extracellular phosphatases	Extracellular phosphatases	Extracellular phosphatases	0	–	+
Nitrogen mineralization	—	Proteases, chitinases	—	0	+	–
Symbiotic nitrogen fixation	Symbioses with *Burkholderia*		Symbioses with *Rhizobium, Frankia,* etc.	–	+	–

fungi to forage over relatively long distances for patches of N or P in the soil (Duddridge et al., 1980; Cairney, 1991; Friese and Allen, 1991; Jennings, 1991; Agerer, 1992; Boddy and Watkinson, 1995). Altogether, just as root:shoot ratios are intrinsically greater in plants native to habitats with low soil fertility or high sunlight (Chapin, 1980; Aerts and Chapin, 2000), extraradical:intraradical ratios should be higher in mycorrhizal fungi that dominate under elevated CO_2, especially where soil fertility is otherwise low.

The low C and low N/P mycorrhizal groups should also differ in traits related to nutrient storage (Table 36.1). When a particular nutrient is often limiting in an environment, economic theory predicts that organisms will take up that nutrient in excess (i.e., luxury consume) whenever it is abundant. The nutrient will be stored for future use during times of scarcity (Bloom et al., 1985). Luxury consumption by plants is more often exhibited by species adapted to low-nutrient environments (Chapin, 1980). Similarly, AM fungi in the low C group may be more likely to store any temporary excess C as lipids in vesicles. Likewise, low P fungi may store extra P as polyphosphate in root sheaths (ECM) or hyphae (AM) (Boddington and Dodd, 1999; Lussenhop and Fogel, 1999).

Mycorrhizal fungi are able to influence rates of nutrient supply in the soil via mineralization, and possibly through symbioses with N-fixing bacteria. Low N/P species might invest more in this activity than would low C species (Table 36.1). Specifically, both AM and ECM fungi produce extracellular phosphatases, which release phosphate from organic material in the soil (Ho and Zak, 1979; Dighton, 1983; Dodd et al., 1987; Joner and Johansen, 2000; Joner et al., 2000). Ectomycorrhizal fungi can also secrete proteases and chitinases to increase N supply (Lundeberg, 1970; Leake and Read, 1990, 1997; Hodge et al., 1995). Greater intrinsic production of extracellular phosphatases should occur in low P species, and of proteases and chitinases in low N species (Lilleskov et al., 2002b). In addition, because enzymes are N-rich compounds, they can represent a significant investment of N (Treseder and Vitousek, 2001). As such, extracellular phosphatase production may be relatively reduced in low N fungi (Olander and Vitousek, 2000; Treseder and Vitousek, 2001). While AM fungi are not known to produce extracellular enzymes that mineralize N, some groups harbor endosymbiotic *Burkholderia* bacteria that appear to fix N from atmospheric N_2 (Bianciotto et al., 1996, 2000; Minerdi et al., 2001, 2002; Bianciotto and Bonfante, 2002). These symbionts might form an important source of N for AM fungi, although the extent to which N fixation occurs has not yet been quantified. If the symbiotic relationship between AM fungi and *Burkholderia* is similar to that between legumes and *Rhizobium*, then the fungi may supply the bacteria with carbohydrates in exchange for N. If this is the case, low N species should be more likely to harbor *Burkholderia* than would low C species. In addition, low P availability inhibits N fixation (Vitousek and Howarth, 1991), so low P species may not readily form relationships with *Burkholderia*. In general, low N or low P species might be particularly likely to contribute to mineralization of organic matter, or to facilitate the input of N into ecosystems through N fixation. If they become more prevalent under elevated CO_2, as hypothesized, large-scale changes in nutrient cycling could result.

The framework presented here is intended to complement previous classifications of fungi by successional stage (Dighton and Mason, 1985; Last et al., 1987; Frankland, 1998; Hart et al., 2001), life history traits (Boddington and Dodd, 1999), generalist vs. specialist qualities (Wallenda and Kottke, 1998), and ecological strategies (i.e., ruderal, competitive, and stress tolerant; Pugh, 1980). For example, according to the successional classification system, mycorrhizal fungi that dominate early in succession are expected to display rapid colonization of plant roots, whereas late-successional species should grow more slowly and compete more effectively for scarce resources (Dighton and Mason, 1985; Hart et al., 2001). In this case, the late-successional group could consist of mycorrhizal

species that also belong to the low N/P category, since soil nutrients are often limiting at later successional stages. Most of these classifications were originally developed for plants (e.g., Clements, 1912; Grime, 1979; Tilman, 1985) and then adapted for fungi.

36.3.1 Functional Groups of Arbuscular Mycorrhizal Fungi

Based on patterns of resource allocation, the hypothesized low C and low N/P functional groups appear to correspond with existing arbuscular mycorrhizal genera (Table 36.2). For example, when species of each genus are grown under similar conditions, *Glomus* (Glomeraceae) tends to have greater abundance of arbuscules (Klironomos et al., 1998) and vesicles (Klironomos et al., 1998; Dodd et al., 2000), and lower incidence of external hyphae (Abbott and Robson, 1985; Dodd et al., 2000; Hart and Reader, 2002), runner hyphae (Dodd et al., 2000), and polyphosphate storage (Boddington and Dodd, 1999) than do the other genera. At this time, *Glomus* does not appear to harbor N-fixing bacteria; although bacteria-like organisms have been observed in *Glomus* structures, Bianciotto et al. (1996) were unable to amplify *Burkholderia* genes from them. In contrast, *Gigaspora* and *Scutellospora* (Gigasporaceae) display traits consistent with tolerance of low N availability. They lack vesicles (Smith and Read, 1997), exhibit well-developed external hyphae networks (Abbott and Robson, 1985; Dodd et al., 2000; Hart and Reader, 2002) and conspicuous runner hyphae (Dodd et al., 2000), and form symbioses with *Burkholderia* (Bianciotto et al., 1996, 2000; Minerdi et al., 2001, 2002; Bianciotto and Bonfante, 2002). Finally, *Acaulospora* (Acaulosporaceae) may be particularly competitive in low P soils, given its extensive, fine hyphal branching (Dodd et al., 2000), high extracellular phosphatase activity compared with Glomeraceae (Vosatka and Dodd, 1998), and lack of *Burkholderia* endosymbionts (Bianciotto et al., 1996). (The recently delineated families of Diversisporaceae *fam. ined.*, Paraglomeraceae, and Archaeosporaceae [Redecker, 2000, 2002; Morton and Redecker, 2001; Schussler et al., 2001; Schwarzott et al., 2001; Schussler, 2002] contain too few described species to form generalizations regarding nutrient relations, so these families will not be examined with this chapter.) According to these assignments of AM genera to functional groups, I predict community composition to shift away from *Glomus* and toward *Gigaspora*, *Scutellospora*, and *Acaulospora* under CO_2 enrichment.

36.3.2 Functional Groups of Ectomycorrhizal Fungi

Ectomycorrhizal fungi are polyphyletic, host specific, and relatively diverse, so I do not necessarily expect functional groups to fall along taxonomic lines. Rather, changes in species composition under elevated CO_2 may be evident as alterations in morphology or ecophysiology of the dominant types.

36.3.3 Functional Groups of Saprotrophic Fungi

Plant-based models of nutrient acquisition strategies are less easily applied to saprotrophic fungi because this group can potentially acquire C, N, and P from one source: dissolved organic matter in the soil. Specifically, saprotrophic fungi release extracellular enzymes into soil solution to break down organic material into smaller components such as amino acids and mineral nutrients. The products are then transferred across the fungal membrane. The fungi oxidize C in organic compounds for energy gain. Any N contained within these compounds is often released because N is usually bound directly to C (McGill and Cole, 1981). The mineralized N is either assimilated or released into soil solution. A net release of N will occur if the fungi are C limited, but not if fungi are N limited (Schlesinger, 1997; Chapin et al., 2002). In contrast, P is usually bound to organic C via an ester bond, which can be hydrolyzed and released as phosphate by extracellular phosphatases without

Table 36.2 Nutrient-Related Traits of Three Common Families of Arbuscular Mycorrhizal Fungi

Family	**Glomeraceae**	**Gigasporaceae**		**Acaulosporaceae**	
Genus	***Glomus***	***Gigaspora***	***Scutellospora***	***Acaulospora***	**References**
Arbuscules or hyphal coils	++	+	+	+	Klironomos et al., 1998; van Aarle et al., 2002a
External hyphae	+	++	++	++	Klironomos et al., 1998; Dodd et al., 2000
Fine hyphal branching	+	++	++	+++	Dodd et al., 2000
Runner hyphae	+	++	++	+	Dodd et al., 2000
Vesicles	++	–	–	+	Klironomos et al., 1998; Dodd et al., 2000
Polyphosphate accumulation	–	+	n.d.	n.d.	Boddington and Dodd, 1999; Solaiman et al., 1999
Extracellular acid phosphatases	+	+	++	++	Vosatka and Dodd, 1998; van Aarle et al., 2002b, 2002b
Symbioses with *Burkholderia*	–	+	+	–	Bianciotto et al., 1996, 2000; Minerdi et al., 2001, 2002; Bianciotto and Bonfante, 2002
Functional group	Low C	Low N	Low N	Low P	

Note: n.d. = not determined; – = uncommon or not observed; + = observed; ++ = relatively abundant; +++ = most abundant.

breaking C bonds. Therefore, C and N acquisition are linked for saprotrophic fungi, but P uptake can occur independently (McGill and Cole, 1981).

In each case, though, extracellular enzymes are the mechanism for nutrient gain. These represent an investment of N. If saprotrophic fungi are C or P limited, economic principles of resource allocation suggest that the production of extracellular enzymes should increase. In particular, extracellular phosphatases (in the case of P limitation) or extracellular enzymes broadly targeting C-rich molecules (in the case of C limitation) should be released. Conversely, N-limited saprotrophic fungi may produce fewer, but more specific, extracellular enzymes targeted for N-rich molecules, even if little energy can be derived from them. Such molecules include chitin and lignin. Field and laboratory studies have indicated that extracellular phosphatase activity increases under P-limited conditions (Conn and Dighton, 2000; Olander and Vitousek, 2000; Treseder and Vitousek, 2001), while chitinase and ligninolytic enzyme activities increase when N is limiting (Keyser et al., 1978; Reid, 1979; Olander and Vitousek, 2000). Nitrogen-limited fungi also may invest C in construction of rhizomorphs in order to forage for N-rich patches. Transfers of nutrients to fresh litter from underlying soil horizons — presumably via rhizomorphs — have been documented (Boddy and Watkinson, 1995; Chadwick et al., 1998).

Saprotrophic fungi could potentially be divided into nutrient-related functional groups based on the type and quantity of extracellular enzymes secreted, as well as the formation of rhizomorphs. For example, white-rot fungi, which primarily belong to the Basidiomycota, actively degrade lignin and may be particularly competitive in low N environments. Likewise, rhizomorph formation is more common in basidiomycetes than in other taxa (Smith and Read, 1997). Given that CO_2 enrichment raises the lignin:N ratio of plant litter, and may stimulate plant competition with microbes for N, basidiomycetes may become more prevalent than other groups.

36.4 SHIFTS IN FUNGAL COMMUNITIES UNDER ELEVATED CO_2

36.4.1 Arbuscular Mycorrhizal Communities

Based on nutrient-related traits of fungal groups, I hypothesized that low N/P fungi should respond more positively to elevated CO_2 than low C fungi, resulting in a shift in community composition toward low N/P species. Specifically, *Glomus* is predicted to decrease in abundance relative to other AM genera (Table 36.2). In a chaparral-based study with closed CO_2 chambers, Treseder et al. (2003) found that the distribution of AM genera was fairly even under ambient CO_2, but was dominated by *Scutellospora* and *Acaulospora* under higher CO_2 concentrations. Hyphal lengths of *Glomus* and *Gigaspora* did not change significantly across CO_2 treatments. Likewise, Insam et al. (1999) reported a decline in fitness of smaller-spored (<20 μm), but not larger-spored (>45 μm), AM species within a model tropical ecosystem. (*Glomus* species produce spores within the <20 μm size class, while those of the other genera tend to be larger.) Wolf et al. (2003) characterized AM spore communities in the BioCON experiment at Cedar Creek, MN. Although 11 species were identified, only 2 were affected by CO_2 enrichment: *Glomus clarum* and *Glomus fasciculatum*. They increased and decreased in abundance, respectively, with no change in species richness overall. Although results from the chaparral and tropical studies were fairly consistent with our prediction, those of the BioCON experiment were not. Few published data regarding shifts in AM communities under elevated CO_2 are available at this time. Hopefully, as more information is gathered, stronger patterns will emerge.

Relationships between AM community structure and N availability — even in the absence of CO_2 treatments — provide an additional test of the characterizations of AM functional groups. Low N groups should become less abundant under N fertilization or deposition (Table 36.2), and field studies to date are consistent with this prediction. For example, in an N deposition gradient in southern California, *Scutellospora* and *Gigaspora* were replaced by *Glomus* species as soil N availability increased (Egerton-Warburton and Allen, 2000). In addition, *Glomus* abundance rose as soil fertility increased along a chronosequence in Hawaiian tropical rain forest, and *Scutellospora* was significantly rarer in N-fertilized plots than in P-fertilized plots in the same sites (Treseder and Allen, 2002). Likewise, *Acaulospora* and *Scutellospora* were more prevalent earlier in old-field succession in Minnesota when soil fertility was low, while the opposite was true for *Glomus* (Johnson et al., 1991). Nitrogen fertilization also increased abundance of *Glomus* spores in a *Populus tremuloides* system, but had no effect on *Acaulospora* spores (Klironomos et al., 1997a). Both *Glomus mosseae* and *Gigaspora gigantea* proliferated under N fertilization in tallgrass prairie (Eom et al., 1999). The consistently positive relationship between *Glomus* abundance and N availability indicates that this genus may be particularly adapted to high N conditions relative to other genera, especially *Scutellospora*.

36.4.2 Ectomycorrhizal Communities

Community composition in ECM fungi under elevated CO_2 is expected to shift toward low N/P types with less investment in ECM root sheaths and greater investment in extraradical hyphae and rhizomorphs (Table 36.1). Several studies have documented that morphotypes with more extensive mycelia (Godbold and Berntson, 1997; Godbold et al., 1997; Rey and Jarvis, 1997; Cairney and Meharg, 1999) and thinner sheaths (Kasurinen et al., 1999) can become more common under CO_2 enrichments. In addition, an unknown basidiomycete was more common under higher CO_2 in a *Picea abies* forest (Fransson et al., 2001). Conversely, *Cenococcum*, which belongs to the Ascomycota and does not produce rhizomorphs, declined in abundance in seedlings of *Pinus echinata* and *Quercus alba* (O'Neill et al., 1987). Nevertheless, changes in morphotype frequency may reflect changes in phenotypes, and not necessarily genotypes, of fungi; such results should be interpreted with caution. Other studies have reported no detectable alterations in the community composition of morphotypes or species (Markkola et al., 1996; Rygiewicz et al., 2000).

Community responses to elevated CO_2 can be contrasted with responses to N availability. Changes in ECM diversity and species composition under N additions have been relatively well examined, and recent studies have integrated laboratory and field work in an attempt to relate species abundance to function under various N regimes (Lilleskov and Bruns, 2001). In general, ECM diversity has declined in regions exposed to N deposition or fertilization (Arnolds, 1988, 1991; Karen and Nylund, 1997; Taylor et al., 2000; Lilleskov et al., 2001, 2002b). Specifically, ECM fungi that can use organic N (presumably due to production of extracellular enzymes) tend to dominate at low N availability, while those that cannot tend to proliferate after N addition (Abuzinadah and Read, 1986; Sagara, 1992; Lilleskov et al., 2001, 2002a, 2002b). This trend is consistent with the classification scheme of nutrient-related functional groups (Table 36.1).

Nitrogen responses of ECM fungi are occasionally — but not always — consistent among genera. Groups that are more tolerant of high N availability and cannot degrade protein often include the basidiomycetes *Paxillus involutus* (Laiho, 1970; Arnebrant, 1994; Lilleskov et al., 2001, 2002a), *Laccaria* spp. (Abuzinadah and Read, 1986; Sagara, 1992; Lilleskov et al., 2001), and *Lactarius* spp. (Arnolds, 1988, 1991; Lilleskov et al., 2001, 2002a). These fungi may correspond to the low C or low P functional groups (Table 36.1).

In contrast, ECM fungi that become less abundant under N additions, and that can use organic N sources, consist of *Suillus* spp. (Abuzinadah and Read, 1986; Arnolds, 1988, 1991), *Tricholoma* spp. (Arnolds, 1988, 1991; Lilleskov et al., 2001), and *Cortinarius* spp. (Arnolds, 1988, 1991; Lilleskov et al., 2001, 2002a), which are basidiomycetes, and *Cenococcum geophilum* (Abuzinadah and Read, 1986; Lilleskov et al., 2002a, 2002b), which is an ascomycete. These are potential low N types (Table 36.1). Low N and low C/P groups are each represented in *Hebeloma* (Sagara, 1992; Lilleskov et al., 2001), *Russula* (Arnolds, 1988, 1991; Lilleskov et al., 2001), *Rhizopogon* (Abuzinadah and Read, 1986; Sagara, 1992), and *Tomentella* (Lilleskov et al., 2002a), all basidiomycetes. The low N and low C/P groups overlap in species composition with the late- and early-stage fungi, respectively, that have been identified by successional status (Dighton and Mason, 1985; Arnolds, 1991). The low N functional group might be expected to respond positively to CO_2 enrichment if N becomes limiting. However, *Cenococcum* declined under elevated CO_2 (O'Neill et al., 1987) as well as N deposition (Lilleskov et al., 2002a., 2002b). Few other ECM species have been examined under both conditions.

36.4.3 Saprotrophic Communities

Changes in saprotrophic communities of fungi under CO_2 enrichment have been tracked in a few studies with a variety of approaches, including molecular characterizations, phospholipid fatty acid analyses (PLFAs), and C metabolism assays. To date, Jones et al. (1998) found that elevated CO_2 altered the relative abundance of fungal species, and they also determined that cellulytic decomposer fungi responded more positively to CO_2 than did other groups. However, Zak et al. (2000a) and Klamer et al. (2002) detected no significant effects while applying PLFA and restriction fragment length polymorphism (RFLP) approaches, respectively. Additionally, changes in litter chemistry under elevated CO_2 did not affect the outcome of competition between decomposer fungi (Conway et al., 2000). The diversity of analytical methods employed, in combination with the rich local and regional diversity of species within the saprotrophic guild and paucity of studies, limits my ability to identify general patterns of CO_2 effects among potential functional groups at this time. Investigations of the nutritional dynamics of saprotrophic fungi under numerous environmental conditions constitute a promising area of future research.

36.5 SUMMARY

I have presented here a framework for dividing fungi into functional groups based on nutrient relations, in the hope that this approach will assist investigators in assessing dynamics of fungal communities under elevated CO_2. Preliminary evidence suggests that AM genera may sort into C-, N-, and P-limited types based on ecophysiology and response to environmental conditions. Ectomycorrhizal fungi might also form different functional groups, although their relatively high diversity requires equally diverse investigations of this issue. Saprotrophic fungi are the least understood group within this context. If additional evidence supports this classification system, ecosystem-level consequences of community shifts could potentially be inferred based on suites of fungi traits. For example, elevated CO_2 could indirectly elicit an increase in N fixation if *Gigaspora* and *Scutellospora* proliferate. In addition, because *Gigaspora* and *Scutellospora* can produce more glomalin than *Glomus* (Wright and Upadhyaya, 1999; K.M. Turner, unpublished data), shifts toward the former may have contributed to observed increases in glomalin concentrations in the soil (Rillig et al., 1999, 2000, 2001). By examining relationships between functional groups and environmental conditions, we may also gain insight regarding

controls over microbial biodiversity (Bruns, 1995). As large-scale CO_2 effects are tested in an expanding array of habitats, collaborations between biogeochemists and microbial ecologists should provide valuable opportunities to delineate functional groups of fungi and examine their roles within the ecosystem. The importance of plant species in mediating ecosystem responses to global change has received much attention of late (e.g., Chapin, 1993; Hobbie, 1992); it is logical and timely to apply a similar approach to the fungal community.

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37

Toxic Metals and Fungal Communities

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37.1 INTRODUCTION

Of the major groups of soil organisms, fungi have fundamental roles in nutrient decomposition, animal and plant pathogenesis and symbiosis, and element cycling, as well as a role in maintenance of soil structure due to their filamentous branching growth habit and frequent exopolymer production. It seems obvious that toxic metals and other pollutants will adversely affect soil properties if pollutant concentration and speciation result in mycotoxicity. Like other microorganisms, fungi possess a variety of properties that can influence interactions with metals, while normal growth and metabolism are also dependent on metal and metal–mineral interactions to satisfy trace metal and associated nutrient requirements. Nevertheless, at potentially toxic metal concentrations, a variety of resistance mechanisms may be expressed: sensitive organisms may be vulnerable to toxic symptoms, resulting in population changes. However, metal toxicity can be greatly influenced by the physicochemical attributes of the soil environment, while fungi possess a variety of intrinsic properties that can ensure survival. It seems fungi can be isolated from any soil polluted by toxic metals. This chapter outlines the effects toxic metals may have on fungal communities, the physiogical and morphological strategies employed to combat metal stress, mechanisms of resistance, fungal-mediated metal transformations, and the role of fungi in the geochemistry of metal cycling, as well the applied significance of these processes in environmental biotechnology.

37.2 FUNGAL COMMUNITIES IN METAL-POLLUTED SOILS AND METAL-RICH ENVIRONMENTS

Anthropogenic activities, including fossil fuel combustion, mineral mining and processing, and production of industrial effluents and sludges, biocides, and preservatives, release a variety of toxic metal species into aquatic and terrestrial ecosystems, and this can have significant effects on the biota as well as result in metal transfer to higher organisms, plants and animals (Gadd and Griffiths, 1978; Gadd, 1992b; Wainwright and Gadd, 1997). Restoration of metal-contaminated environments requires a functional microbial community for successful plant community establishment, soil development, and biogeochemical cycling. Many studies on the effects of toxic metals on soil microorganisms have revealed that metal toxicity reduced microbial numbers and activity and greatly affected microbe-mediated processes in soil ecosystems, such as organic matter decomposition and nitrification (Brookes and McGrath, 1984; Chander and Brookes, 1991; Aoyama and Nagumo, 1997a, 1997b; Kuperman and Carreiro, 1997; Olayinka and Babalola, 2001). Because metal-tolerant microorganisms have a competitive advantage among the microbial community, the frequency of tolerant microorganisms may increase with an increase in the toxic metal levels in the soil (Olson and Thornton, 1982; Huysman et al., 1994; Kunito et al., 1997). This can lead to a decrease in species diversity and therefore a shift in microflora composition (Pennanen et al., 1996), which can result in a reduction of soil functional activity. In the case of fungi, metal resistance is genetically inherited as species- or strain-specific characteristics, and adaptation to metal stress is probably of minor importance. Resistant fungal species are usually present at low frequencies in noncontaminated soils but can become dominant under toxic metal stress of heavy metals (Kunito et al., 1998). However, fungi, including ectomycorrhizal (ECM) fungi, show considerable interspecific responses to toxic metals, yet the extent to which intraspecific (adaptive) resistance exists remains unclear (Meharg and Cairney, 2000). It should be mentioned that resistance and tolerance are arbitrarily defined, frequently interchangeable terms, and are often based on whether particular strains can grow in the presence of selected toxic metal concentrations in laboratory media. It is probably more appropriate to use *resistance* to describe a direct mechanism resulting from metal exposure, e.g., metallothionein synthesis. *Tolerance* may rely on intrinsic biochemical and structural properties of the host, such as possession of impermeable cell walls, extracellular slime layers or polysaccharide, and metabolite excretion, as well as environmental modification of toxicity. However, distinctions are difficult in many cases because several direct and indirect mechanisms, both physicochemical and biological, can contribute to survival. Thus, although metal pollution can qualitatively and quantitatively affect microbial populations in the environment, it may be difficult to distinguish metal effects from those of environmental components, environmental influence on metal toxicity, and the nature of the microbial resistance/tolerance mechanisms involved (Gadd, 1992a).

Although bacteria are commonly studied in the context of metal interactions and responses, mycelial fungi can develop a significantly higher biomass and may sequester greater amounts of metals (Massaccesi et al., 2002). Numerous studies have shown that microbial population responses to toxic metals are characterized by a shift within the population from bacteria and streptomycetes to fungi (Mineev et al., 1999; Chander et al., 2001a, 2001b; Kostov and Van Cleemput, 2001; Olayinka and Babalola, 2001; Khan and Scullion, 2002). This can lead to increased decomposition of organic matter and reduced assimilation of released N. For example, the use of sludges with high metal (Cd, Cu, Ni, Pb, or Zn) concentrations led to short-term changes in soil microbial communities and

their activities, with increased loss of C to the atmosphere and N availability (Khan and Scullion, 2002).

All nutritional groups of fungi (saprotrophs, biotrophs, and necrotrophs) are affected by toxic metals. Biotrophic mycorrhizal fungi form complex communities in the root systems of most plant species and are highly important in terrestrial ecosystem sustainability. A relative decrease in an indicator fatty acid for arbuscular mycorrhizal (AM) fungi and an increase for other fungi have been reported in zinc-polluted soil (Kelly et al., 1999). Toxic metals (Cd, Cr, Cu, Ni, Pb, and Zn) contamination of soil led to a significant decrease in the number of arbuscular mycorrhizal fungi and low colonization of plant roots, and as a result to changes in the species diversity of mycorrhizal fungi (Moynahan et al., 2002; Mozafar et al., 2002). Toxic metals also reduce plant root colonization by ectomycorrhizal fungi; e.g., pine seedlings could not form ectomycorrhiza on acidic metalliferous mine spoil (Avoca, Ireland) highly contaminated with copper and lead (Fay and Mitchell, 1999). Multiple contamination with Cd, Pb, Zn, Sb, and Cu as well as Ni deposition in soil had a toxic effect on ectomycorrhizal fungi associated with Scots pine seedlings and caused shifts in the ectomycorrhizal species composition (Hartley et al., 1999; Markkola et al., 2002). The extent to which such changes in the structure of belowground communities of mycorrhizal fungi are sustained in the longer term is not clear, and the major limitation to predicting the consequences of pollution-mediated changes in mycorrhizal fungal communities is the limited understanding of the functional significance of mycorrhizal biodiversity (Cairney and Meharg, 1999).

A study of cadmium effects on potentially pathogenic soil fungi showed that non-keratinolytic fungi showed a higher tolerance to Cd than keratinolytic fungi (Plaza et al., 1998). Zygomycetes were found to be more tolerant to Cd than ascomycetes and deuteromycetes (Plaza et al., 1998). The most frequent soil saprotrophic microfungi isolated from heavily metal polluted habitats in Argentina, the Czech Republic, and the Ukraine were reported to be species of *Penicillium*, *Aspergillus*, *Trichoderma*, *Fusarium*, *Rhizopus*, and *Mucor*, as well as *Paecilomyces lilacinus*, *Nectria invertum*, *Cladosporium cladosporioides*, *Alternaria alternata*, and *Phoma fimeti* (Kubatova et al., 2002; Massaccesi et al., 2002; Fomina, Manichev, Kadoshnikov, and Nakonechnaya, unpublished). The melanized fungi of the Dematiaceae family (species of genus *Cladosporium*, *Alternaria alternata*, and *Aureobasidium pullulans*) were often isolated from soil samples treated with toxic industrial wastes containing high concentrations of copper and mercury (Zhdanova et al., 1986) and may also be dominant members of the mycoflora of metal-contaminated phylloplanes (Mowll and Gadd, 1985). Dark septate endophytes were found to be the dominant fungi among isolates from healthy fine roots of *Erica herbacea* L. in Pb-, Cd-, and Zn-polluted soil (Cevnik et al., 2000). Strains of *Aspergillus* were more tolerant to cadmium than strains of *Penicillium* (Plaza et al., 1998). However, *Penicillium* species are often reported to be dominant in copper-contaminated environments. In Brazilian soils with copper concentrations of 25 to 11,500 mg/kg, the predominance of a *Penicillium* species tolerant to a copper concentration of 750 mg/kg in soil was found (Ribeiro et al., 1972). It was also observed that there was a very high predominance of *Penicillium* species (80% of isolations) in freshly excavated archeological soil, containing 500 mg/kg copper and 500 mg/kg lead, at the site of an ancient Greek copper-smelting furnace (the Bronze Age, ancient Greek colony, Olviya city, Southern Ukraine; Fomina, Manichev, Kadoshnikov, and Nakonechnaya, unpublished). One of the most useful bioindicators for soil contamination and ecotoxicity of toxic metals in soil is the ratio of metal-tolerant to metal-sensitive microorganisms (Kunito et al., 1998). For example, the ratio of copper-tolerant to copper-sensitive fungal isolates was 50 to 92% in different toxic metal-polluted soils and only 2

to 19% in nonpolluted soils from southern Ukraine (Fomina, Manichev, Kadoshnikov, and Nakonechnaya, unpublished).

Investigations on metal toxicity in mycorrhizal, especially ectomycorrhizal, fungi at a species and community level have revealed wide inter- and intraspecific variations in metal sensitivity (Jones and Muehlchen, 1994; Hartley et al., 1997; Vodnik et al., 1998; Blaudez et al., 2000, Meharg and Cairney, 2000). For fungi growing on wood at metal-contaminated sites, natural selection for metal-tolerant strains was not observed (Baldrian and Gabriel, 2002; Baldrian, 2003). However, the situation may be different in soil where the concentrations of toxic metals could be considerably higher, and the process of adaptation to metal stress is probably accompanied by the exclusion of metal-sensitive fungal strains (Baldrian, 2003). Many studies have suggested that selection for resistant ecotypes occurs where the degree of toxic metal contamination and selection pressure is high (Colpaert et al., 2000; Sharples et al., 2000, 2001). For example, isolates of the ericoid mycorrhizal fungus *Oidiodendron maius*, recovered from *Vaccinium myrtillus* growing in metal-polluted areas, were in general less sensitive to metals than strains from nonpolluted sites (Lacourt et al., 2000). *In vitro* Zn tolerance of isolates of the ectomycorrhizal fungus *Suillus luteus* from a zinc-polluted habitat was significantly higher than isolates from a nonpolluted site (Colpaert et al., 2000). An isolate of the ectomycorrhiza *Pisolithus* sp. from a chromium and nickel-contaminated site not only colonized a greater percentage of root tips *in vitro*, but was more effective in promoting *Eucalyptus urophylla* seedling total biomass in Ni-amended soils than the other isolates (Aggangan et al., 1998). Undoubtedly, metal effects on natural soil communities are complex and difficult to characterize because of the complex array of contributing factors. Many contaminated sites contain mixtures of metals as well as organic pollutants, and each may have reciprocal physical and chemical effects on the other with complexation and other phenomena affecting toxicity, bioavailability, and degradation, for example.

Another ecological metal-rich niche inhabited by fungi is the rock environment, which often contains toxic metal minerals. Fungi have been reported from a wide range of rock types and building stone, including limestone, soapstone, marble, granite, sandstone, andesite, basalt, gneiss, dolerite, amphibolite, quartz, and cement, even from the most harsh environments, e.g., hot and cold deserts (Staley et al., 1982; Gorbushina et al., 1993; Sterflinger, 2000; Verrecchia, 2000) (Figure 37.1). However, it is likely that they are ubiquitous components of the microflora of all rocks and building stone, occurring over a wide range of geographical and climatic zones. Fungi often make up a significant component of epilithic, endolithic, chasmolithic, cryptoendolithic, or euendolithic microbial communities inhabiting subaerial microenvironments (Staley et al., 1982; Gorbushina et al., 1993; Gerrath et al., 1995; Bogomolova et al., 1998; Kumar and Kumar, 1999; Sterflinger, 2000; Burford et al., 2003a, 2003b) (Figure 37.1). Fungi form a major component of microbial biofilms (an assemblage of surface-associated microbial cells enclosed in an extracellular polymeric substance matrix) in rock and building stone. They can exist as free-living fungi or as lichens (symbiosis between ascomycete or basidiomycete fungi and green algae (mainly *Trebouxia*) or, less often, cyanobacteria. Among the most commonly reported fungal groups inhabiting exposed rock surfaces are the microcolonial fungi (MCF) described by Staley et al. (1982). These are also known as the black yeasts or yeast-like black meristematic fungi (Gorbushina et al., 1993; Wollenzien et al., 1995; Sterflinger, 2000; De Leo et al., 2003). Meristematic growth is characterized by the production of swollen isodiametric cells with thick pigmented (melanin containing) cell walls. In addition to meristematic growth, many of the black fungi exhibit yeast-like stages of reproduction (Gadd, 1980; Gorbushina et al., 1993; Wollenzien et al., 1995; Bogomolova et al., 1998; Sterflinger, 2000; De Leo et al., 2003).

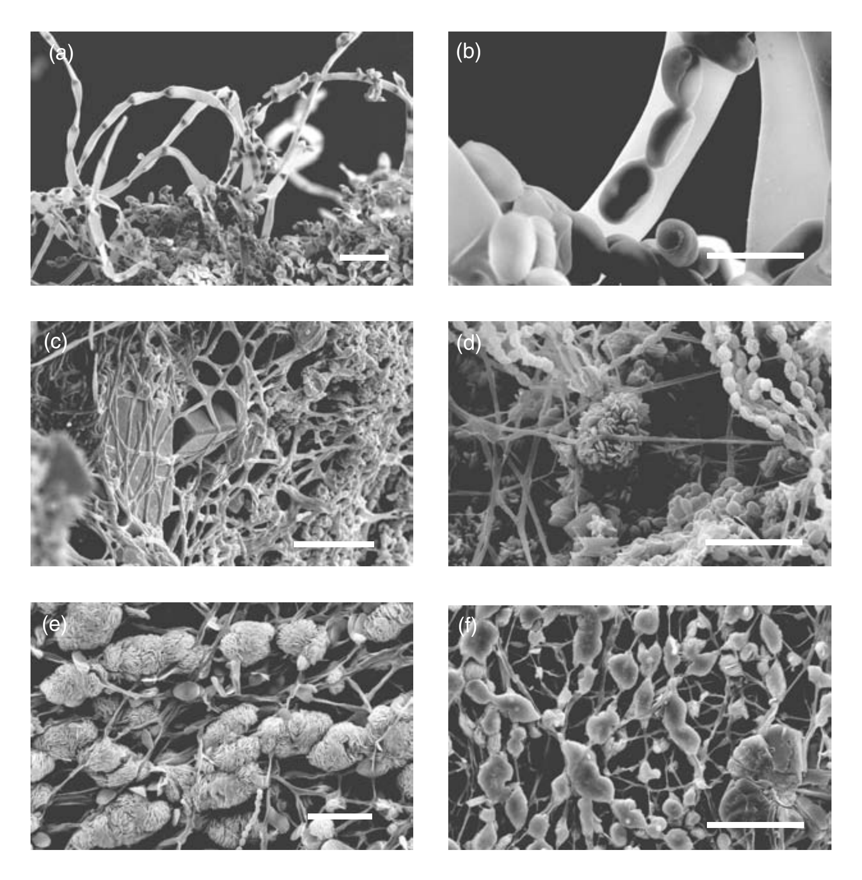


Figure 37.1 Scanning electron microscopy (SEM) images of fungal communities in metal-rich environments. (a–c) Yeast-like and hyphal growth *in situ* on the surface of limestone. (d) Formation of Ca-containing secondary precipitates *in situ* in limestone by *Penicillium simplicissimum*. (e) Formation of secondary precipitates containing Ca and Cu when grown in agar containing 50 m*M* $CaCO_3$ + 5 m*M* cuprite by *P. simplicissimum*. (f) Formation of secondary precipitates containing Ca and Fe when grown in agar containing 50 m*M* $CaCO_3$ + 5 m*M* hematite by *Chaetomium* spp. (a, b) Samples were air dried. (c–f) Au/Pd-coated samples were treated with the vapor diffusion dehydration (acetone) method and Au/Pd coated. Bar markers: 20 μm (a, d, e), 5 μm (b), 10 μm (c), 50 μm (f) (Burford et al., unpublished).

37.3 PHYSIOLOGICAL RESPONSES OF FUNGI TO TOXIC METAL STRESS

Toxic metals can inhibit the growth and spore germination of fungi, affect reproduction and metabolic activity, and reduce the ability of mycorrhizal fungi to colonize roots of host plants (Gadd, 1993a; Amir and Pineau, 1998; Fay and Mitchell, 1999; Hartley-Whitaker et al., 2000; Moynahan et al., 2002; Mozafar et al., 2002; Baldrian, 2003).

37.3.1 Toxicity

The effects of toxic metals on fungal growth have shown intra- and interspecific variability and dependence on metal species and speciation (Gadd, 1993a; Plaza et al., 1998). Nickel was reported to be more toxic to fungal growth and ectomycorrhiza formation than chromium (Aggangan et al., 1998). Copper was found to be toxic and Cd very toxic to

cultures of 15 decomposer basidiomycetes examined using a microwell technique (Hoiland, 1995). However, a similar toxicity of both metals was shown on a solid medium with Cd and Cu reducing radial growth of most strains of aquatic hyphomycetes by 50% at concentrations between 150 and 400 μ*M* (Miersch et al., 1997). Radial extension rates of *Trichoderma virens* did not significantly differ during growth on tap water agar containing glucose and 0.1 m*M* Cu or Cd (Ramsay et al., 1999). For *T. virens* and *Clonostachys rosea* colonizing spatially discrete toxic metal–containing domains, colonization distance, hyphal extension rates, and the efficacy of carbon substrate utilization decreased considerably with increasing concentration of copper and cadmium (Fomina et al., 2003).

It has been observed that a decrease of metal toxicity is correlated with an increase in the concentration of available carbon source (Ramsay et al., 1999; Fomina et al., 2003). For the basidiomycetes *Stereum hirsutum* and *Trametes versicolor* cultivated in the presence of cadmium and mercury, toxicity was lower in rich, complex media (Baldrian and Gabriel, 1997), although metal binding to medium constituents may be a factor in this case (Gadd and Griffiths, 1978). This was also reported for *T. virens* grown on tap water agar media containing glucose (0.5 to 10%) and 0.1 m*M* toxic metals (Cu, Cd, and Zn) where radial extension rate was commensurate with the availability of carbon, revealing a decrease in metal toxicity with increasing levels of available glucose (Ramsay et al., 1999). The tolerance of decomposer basidiomycetes to Cd was shown to be higher in fungi from rich, basophilous soils than from poor, acidic soils, whereas resistance to Al was highest in fungi living in poor, acidic soils (Hoiland, 1995). A decrease of fungal growth rate in the presence of toxic metals is sometimes accompanied by an increase in the lag phase (or growth delay) (Gadd and Griffiths, 1980b; Baldrian and Gabriel, 2002; Baldrian, 2003). A considerable increase in the lag period was observed for *T. virens* and *C. rosea* grown with copper and cadmium (Fomina et al., 2003).

37.3.2 Sporulation and Germination

Toxic metal treatment was reported to reduce the sporulating ability of *Aspergillus niger* and arbuscular mycorrhizal fungi (Magyarosy et al., 2002; Liao et al., 2003). Spore germination was reported to be more sensitive to toxic metals (Ni^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, and Mg^{2+}) than mycelial growth (Amir and Pineau, 1998). However, a proportion of the spores of metal-sensitive fungal isolates of *Curvularia* sp. and *Fusarium* sp. was able to germinate and grow moderately well in the presence of relatively high metal concentrations (Amir and Pineau, 1998).

37.3.3 Enzyme Activity

Toxic metals can be potent inhibitors of enzymatic reactions. Cd, Cu, Pb, Mn, Ni, and Co considerably decreased cellulase and amylase production by several fungi, including yeasts, with reduced enzyme activity correlated with increasing metal concentration (Falih, 1998a, 1998b). It was also suggested that the lack of reactivity of purified laccases from the fungus *Pycnoporus cinnabarinus* toward hydrocarbons could be due to metal interference (Mougin et al., 2002). However, other studies have shown that extracellular laccase activity was significantly stimulated by cadmium in white-rot basidiomycetes (Jarosz-Wilkolazka et al., 2002; Baldrian and Gabriel, 2003). In lignocellulose degradation by *Pleurotus ostreatus*, a decrease in substrate dry weight and Mn-peroxidase activity decreased with increasing Cd concentration, whereas the activities of endo-1,4-β-glucanase, 1,4-β-glucosidase, and laccase were significantly increased in the presence of this metal (Baldrian and Gabriel, 2003). The addition of increasing concentrations of Zn^{2+} and Cd^{2+}, to levels below 1 m*M*, to purified polygalacturonase from a metal-tolerant ericoid mycorrhizal isolate of *Oidiodendron maius* increased enzyme activity, but the same metal

concentrations did not affect or only slightly inhibited extracellular enzyme activity in the nontolerant isolate (Martino et al., 2000).

37.3.4 Melanization in Response to Toxic Metals

A variety of toxic metals can induce or accelerate melanin production in fungi, leading to blackening of colonies and chlamydospore development (Gadd and Griffiths, 1980a). Chlamydospores and other melanized forms have high capacities for metal biosorption, with the majority of metal remaining within the wall structure (Gadd, 1984; Gadd and Mowll, 1985; Gadd et al., 1987; Gadd and De Rome, 1988). In rhizomorphs of *Armillaria* sp. the highest concentrations of metals were located on the melanized outer surface (Rizzo et al., 1992). A significant proportion of fungal biomass in soils is melanic (Bell and Wheeler, 1986), and such interactions may be of ecological significance in polluted habitats (Gadd, 1993a).

37.4 MORPHOLOGICAL STRATEGIES OF MYCELIAL SYSTEMS IN RESPONSE TO TOXIC METAL STRESS

Fungal morphology can be altered by different toxic metals, and changes in mycelial density have often been observed. For example, *Schizophyllum commune*, *Daedalia quercina*, and *Paxillus involutus* exhibited increased hyphal branching in response to cadmium (Darlington and Rauser, 1988; Lilly et al., 1992; Gabriel et al., 1996). *S. commune* also developed loops and connective filaments under cadmium stress (Lilly et al., 1992). Changes in mycelial morphology have also been observed in *S. hirsutum* and *T. versicolor* cultivated in the presence of cadmium and mercury (Baldrian and Gabriel, 1997), in a copper-tolerant *Mucor rouxii* strain in the presence of a high copper concentration (Gardea-Torresdey et al., 1997) and in ectomycorrizal fungi during growth in metal-containing agar plates (Cu, Al, Zn) (Jones and Muehlchen, 1994). It was also found that the biomass distribution of *T. viride* colonies was altered by the presence of toxic metals, with biomass concentrated in the periphery of the colonies in the presence of copper and toward the interior of the colonies in the presence of cadmium (Ramsay et al., 1999; Gadd et al., 2001).

Metal-contaminated soils often contain a spatially heterogeneous distribution of metal concentrations and nutritional resources available, and an experimental system based on tessellated agar tiles, simulating this heterogeneity, has been successfully used for the study of morphological changes of fungi colonizing spatially discrete metal-containing domains. During growth of fungi in metal-containing agar tiles, a wide range of morphological changes and growth responses occurred (Fomina et al., 2000, 2003). In the gap between metal-free and metal-containing tiles, the presence of copper or cadmium led to negative chemotropic reactions in *Geotrichum candidum*, *C. rosea*, *Humicola grisea*, and *T. virens* and cessation of growth, swelling, and lysis of some hyphal tips of *T. virens* and *C. cladosporioides* (Figure 37.2) (Fomina et al., 2000, 2003; Fomina and Gadd, unpublished). Many strategies that fungi employ in physicochemically hostile metal-polluted environments can be thought of as analogous to those adopted in human warfare, and applying this concept, negative tropisms and growth cessation can be viewed as a retreat strategy aimed at avoiding toxic metal-contaminated areas (Figure 37.2 to Figure 37.4). The concept also includes guerrilla and phalanx growth forms of mycelial systems, as well as reallocation strategies by the formation of mycelial cords (aggregation of longitudinally aligned hyphae) (Lovett-Doust, 1981; Boddy, 1993; Carlile, 1995) (Figure 37.3 to Figure 37.5). A single mycelium can adopt guerilla or phalanx strategies as required, although inherent characters of the species may impose limits on such adaptation. Changes

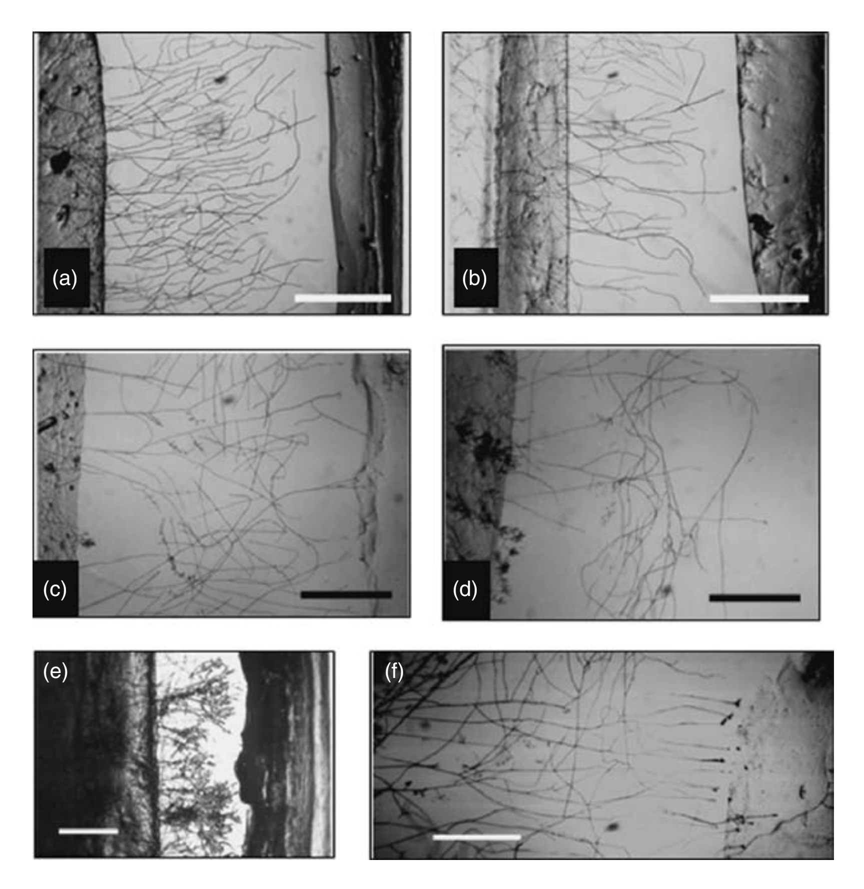


Figure 37.2 Local mycelial responses to copper observed in the gap between a metal-free (inoculum) and copper-containing (metal) domain. (a–b) Negative chemotropic responses by hyphae of *Geotrichum candidum* growing toward copper-containing tiles of agar medium (containing 30 gl^{-1} of sucrose) on the right-hand side of the images containing 2 m*M* Cu (see Fomina et al., 2000). (c, d) Negative chemotropic responses to toxic metals by *Trichoderma virens* growing toward metal-containing tiles of agar medium on the right-hand side: 2 m*M* Cu and 15 gl^{-1} sucrose (c) and 2 m*M* Cu and 1 gl^{-1} sucrose (d) (see Fomina et al., 2000). (e, f) Cessation of growth, swelling, and lysis of hyphal tips of *Cladosporium cladosporiodes* growing toward a metal-containing tile with 1 gl^{-1} sucrose and 1 m*M* Cu (Fomina and Gadd, unpublished). (f) *T. virens* growing toward the metal-containing tile with 1 gl^{-1} sucrose and 2 m*M* Cu on the right-hand side (see Fomina et al., 2003). Bar marker = 0.5 mm.

in these strategies can be represented by changes in branching patterns or different degrees of commitment to radial (explorative) or tangential (exploitative/consolidative) growth (Rayner et al., 1995). Penetration of hyphae into metal-containing domains was often followed by the formation of very dense mycelia or mycelial bushes representing an associative (constraining, exploitative, or phalanx) growth strategy of the mycelial system (Fomina et al., 2003) (Figure 37.3b and f and Figure 37.4). Any hyphal aggregation could be an example of the phalanx growth form, with profusely branching hyphae facilitating the colonization of a substrate, and the production of high local concentrations of extracellular enzymes, antibiotics, and other metabolites. In the case of a toxic metal-containing domain, aggregated mycelia could produce high local concentrations of many extracellular products, such as chelating and sequestering agents (e.g., organic acids, siderophores,

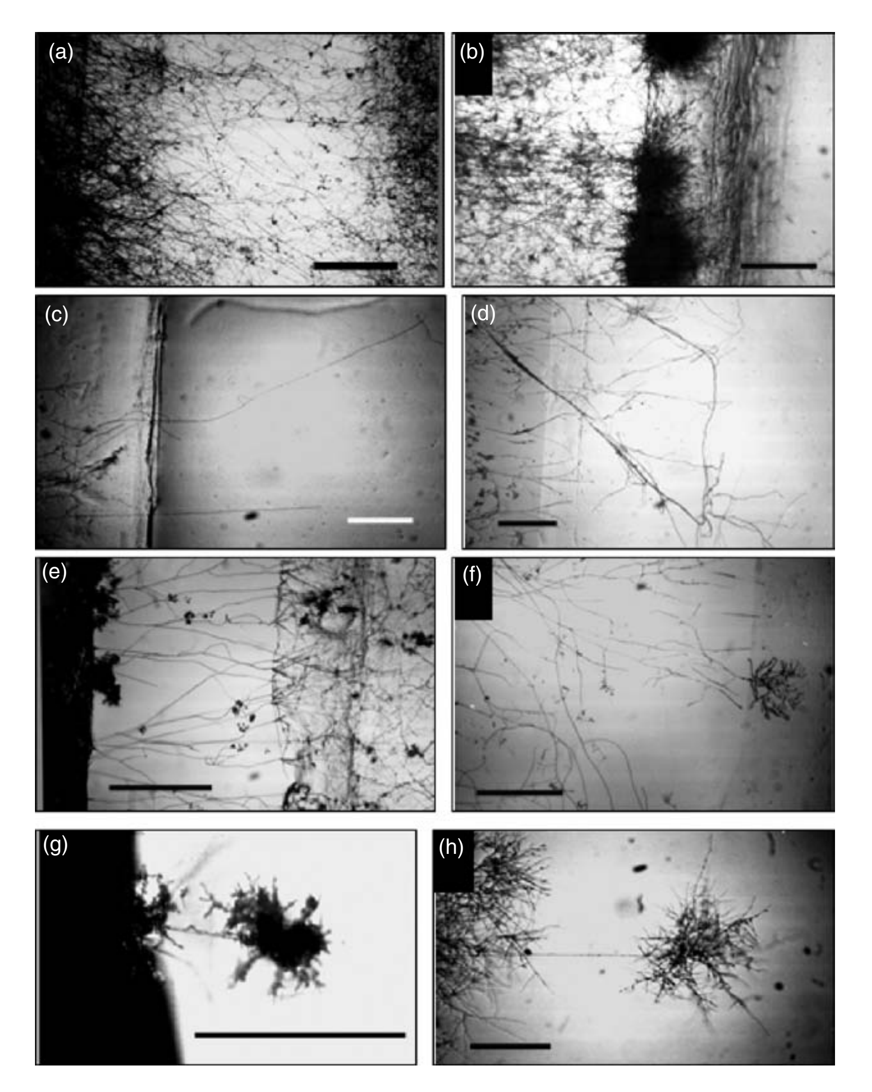


Figure 37.3 Morphological responses of mycelial systems to toxic metal stress when penetrating a metal-containing domain (on the right-hand side of the image). (a–d) *Clonostachys rosea*. (e, f) *Trichoderma virens*. (a, e) Control growth on agar tile with no toxic metal added. (b, f) Mycelial bushes appearing as phalanx or point growth phenomena on the agar tile containing 5 gl^{-1} sucrose and 1 m*M* Cd (b) and 1 gl^{-1} sucrose and 2 m*M* Cd (f). (c, d) Long sparsely branched, branchless, and curling explorative hyphae as guerrilla strategy of the mycelial system on tiles containing 2 m*M* Cu with 15 gl^{-1} sucrose (c) and 5 gl^{-1} sucrose (d). (Adapted from Fomina et al., *Mycological Research*, 107, 861–871, 2003.) (g, h) Characteristic examples of repeated phase shifts showing slow–dense/fast–effuse/slow–dense mycelial transitions during growth of *Cladosporium cladosporioides* (g) and *T. virens* (h) on domains containing 1 m*M* Cu and 1 gl^{-1} sucrose. Bar marker = 0.5 mm. (Adapted from Fomina et al., *Mycological Research*, 107, 861–871, 2003; Fomina et al., unpublished.)

polyphenolic compounds), metal precipitating agents (e.g., oxalate), and polysaccharides and pigments with metal-binding abilities (Gadd, 1993a; Dutton and Evans, 1996; Morley et al., 1996; Baldrian, 2003). The protective role of hyphal aggregation depends on the ability of mycelial systems to shift between assimilative and nonassimilative states, which enables mycelia to penetrate or grow across physicochemically hostile regions, and in

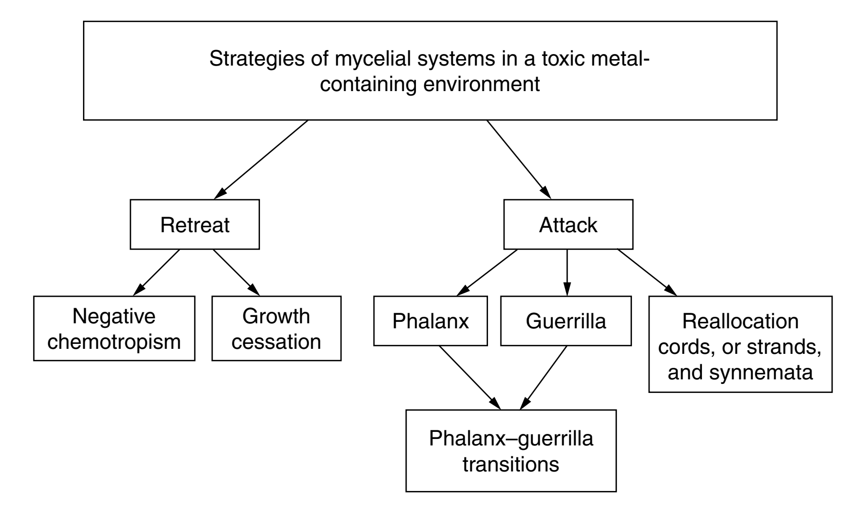


Figure 37.4 Diagram of warfare strategies of mycelial systems in a toxic metal-containing environment.

some cases to ameliorate conditions for superseding exploitative phases (Rayner et al., 1995). After fungi enter toxic metal-containing domains under poor nutritional conditions, they often produced long sparsely branched or branchless explorative hyphae representing a dissociative (expansive, explorative, or guerrilla) growth strategy with a large hyphal growth unit and infrequent branching (Fomina et al., 2003) (Figure 37.3c and d and Figure 37.4). It was also observed that fungi were able to exhibit multiple repeated phase shifts or phalanx–guerrilla–phalanx transitions, e.g., when *T. virens* and *C. cladosporioides* growing on copper at low sucrose concentrations first formed bordering mycelial bushes, then long branchless explorative hyphae, and then bushes on the tips of these exploring hyphae (Figure 37.3g and h and Figure 37.4) (Fomina et al., 2003; Fomina and Gadd, unpublished). It seems that fungi can efficiently use both the phalanx and guerrilla states of the mycelial system and their transitions to resist toxic metal stress combined with poor nutritional conditions. It was also found that microfungi penetrating metal-contaminated domains could form mycelial cords and synnema, which may be atypical for these fungal species under normal conditions, thus combining a protective role of hyphal aggregation with a reallocation strategy (Figure 37.4 and Figure 37.5) (Fomina and Gadd, unpublished). Metal cations are believed to be involved in the formation of synnema of the penicillia (Tinnell et al., 1977). In *Sphaerostilbe repens*, both synnematal and rhizomorph development only occurred in the presence of calcium, with strontium (which often behaves as a calcium ion analogue) also capable of the induction of some morphological changes (Botton, 1978). The production of synnemata results in a wider separation between the conidia and the substrate than in nonsynnematal colonies, and this may aid dispersal as well as ensure conidia formation away from potential toxicants in the substrate (Newby and Gadd, 1987).

37.5 MECHANISMS OF METAL RESISTANCE AND TOLERANCE

Metals and their compounds can interact with fungi in various ways depending on the metal species, organism, and environment, while metabolic activity can also influence

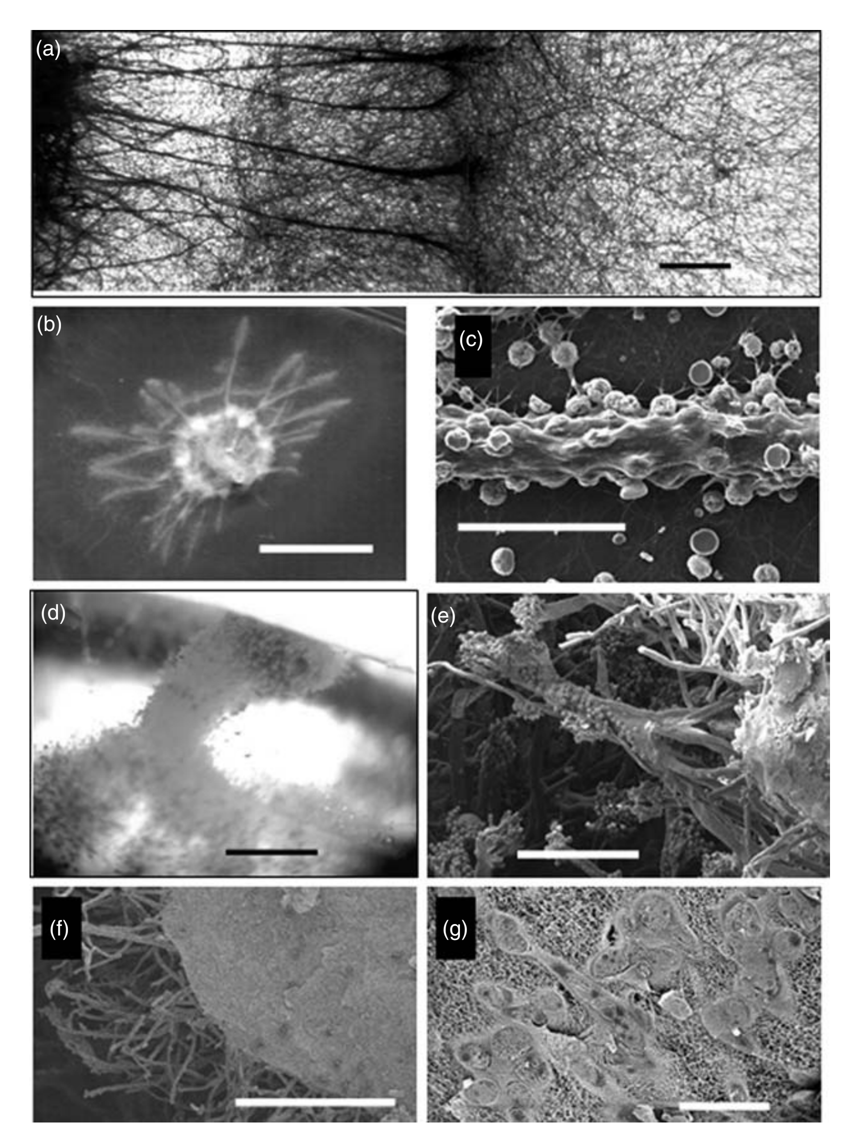


Figure 37.5 **(See color insert following p. 460.)** Unusual structures of aggregated and longitudinally aligned hyphae formed by fungi in response to toxic metal stress. (a) *Cladosporium cladosporioides*: melanized strands formed in the gap between an inoculum domain (Czapek–Doz agar tile) and a metal-containing tile (1 m*M* Sr and 5 gl^{-1} sucrose) on the right-hand side of the image. Bar = 0.5 mm. (b, c) *Beauveria caledonica*: strands formed on modified Melin–Norkrans (MMN) medium containing copper phosphate, covered with copper oxalate crystals. (b) Light microscopy (LM) image of the colony on the membrane. Bar =10 mm. (c) A strand with crystals (Au/Pd-coated air-dried sample). Bar = 200 µm. (d–g) *Aspergillus flavipes*: large synnema (= coremium) covered with conidiophores and synnema, formed between an inoculum domain (Czapek–Doz agar tile) and a metal domain (1 m*M* Cu gl^{-1} sucrose) on the top right-hand side of the image. (d) LM image of large synnema. Bar = 1mm. (e) Cryo-FESEM image of synnema on the large synnema surface. Bar = 50 µm. (f, g) Cryo-field emission electron microscopy (Cryo-FESEM) images of cross-fractured large synnema showing compact hyphae inside expolymer matrix with conidiophores and synnema in the outer shell. Bar = 100 µm (f) and 10 µm (g). (Fomina and Gadd, unpublished.)

speciation and mobility. Many metals are essential for fungal growth and metabolism, e.g., Na, K, Cu, Zn, Co, Ca, Mg, Mn, and Fe, but all can exert toxicity when present above certain threshold concentrations in available forms (Gadd, 1993a). Other metals, e.g., Cd, Hg, Pb, have no known biological function, but all can be accumulated by fungi (Gadd, 1993a). Metal toxicity is greatly affected by the physicochemical nature of the environment and the chemical behavior of the particular metal species in question. Metals exert toxic effects in many ways; e.g., they can block the functional groups of important biological molecules such as enzymes, displace or substitute for essential metal ions, cause disruption of cellular and organellar membranes, and interact with systems that normally protect against harmful effects of free radicals generated during normal metabolism (Gadd, 1992a, 1993a; Avery et al., 1996; Howlett and Avery, 1997). Despite apparent toxicity, many fungi survive, grow, and flourish in apparently metal-polluted locations, and a variety of mechanisms, both active and incidental, contribute to tolerance. Fungi possess many properties that influence metal toxicity, including the production of metal-binding proteins, organic and inorganic precipitation, active transport, and intracellular compartmentalization, while major constituents of fungal cell walls, e.g., chitin, melanin, have significant metal-binding abilities (Gadd and Griffiths, 1978; Gadd, 1993a). All these mechanisms are highly dependent on the metabolic and nutritional status of the organism since this will affect expression of energy-dependent resistance mechanisms as well as synthesis of wall structural components, pigments, and metabolites, which gratuitously affect metal availability and organism response (Gadd, 1992a, 1993a; Ramsay et al., 1999).

Fungi are able to restrict metal entry into cells by (1) reduced metal uptake or increased metal efflux; (2) metal immobilization, e.g., cell wall adsorption, extracellular precipitation of secondary neoformed minerals (e.g., oxalates); and (3) extracellular metal sequestration by exopolysaccharides and other extracellular metabolites (Gadd, 1993a, 2001a, 2001b; Macreadie et al., 1994; Blaudez et al., 2000; Perotto and Martino, 2001; Baldrian, 2003). Metal-tolerant fungi can survive due to their ability for intracellular chelation, e.g., by metallothioneins and phytochelatins and metal sequestration within vacuoles. The fungal vacuole has an important role in the regulation of cytosolic metal ion concentrations and the detoxification of potentially toxic metal ions (White and Gadd, 1986; Gadd, 1993a; Gharieb and Gadd, 1998; Liu and Culotta, 1999). Metals preferentially sequestered by the vacuole include Mn^{2+} (Okorokov et al., 1985; Gadd and Laurence, 1996), Fe^{2+} (Bode et al., 1995), Zn^{2+} (White and Gadd, 1987), Co^{2+} (White and Gadd, 1986), Ca^{2+} and Sr^{2+} (Okorokov et al., 1985; Borst-Pauwels, 1989; Gadd, 1993a; Okorokov, 1994), Ni^{2+} (Joho et al., 1995), and the monovalent cations K^+, Li^+, and Cs^+ (Okorokov et al., 1980; Perkins and Gadd, 1993a, 1993b). The absence of a vacuole or a functional vacuolar H^+-ATPase in *Saccharomyces cerevisiae* is associated with increased sensitivity and a largely decreased capacity of the cells to accumulate Zn, Mn, Co, and Ni (Ramsay and Gadd, 1997), metals known to be mainly detoxified in the vacuole (Gadd, 1993a; Joho et al., 1995).

For Cu and Cd, intracellular detoxification appears to predominantly depend on sequestration in the cytosol by induced metal-binding molecules (Hayashi and Mutoh, 1994; Macreadie et al., 1994; Ow et al., 1994; Rauser, 1995). These include low-molecular-weight cysteine-rich proteins (metallothioneins) and peptides derived from glutathione (phytochelatins) (Mehra and Winge, 1991; Macreadie et al., 1994; Ow et al., 1994; Rauser, 1995; Wu et al., 1995; Inouhe et al., 1996). The latter peptides have the general structure of $(\gamma\text{Glu-Cys})_n$-Gly where the γGlu-Cys repeating unit may extend up to 11 (Ow et al., 1994). In *Schizosaccharomyces pombe* the value of n ranges from 2 to 5, while in *S. cerevisiae*, only an n_2 isopeptide has been observed (Macreadie et al., 1994). As well as being termed phytochelatins, such peptides are also known as cadystins and metal γ-glutamyl peptides, although the chemical structure, $(\gamma EC)_nG$, is an alternative description.

Although $(\gamma EC)_nG$ induction has been reported with a wide variety of metal ions, including Ag, Au, Hg, Ni, Pb, Sn, and Zn, metal binding has been shown for only a few, primarily Cd and Cu (Ow et al., 1994). For Cd, two types of complexes exist in *S. pombe* and *Candida glabrata*. A low-molecular-weight complex consists of $(\gamma EC)_nG$ and Cd, whereas a higher-molecular-weight complex also contains acid-labile sulfide (Murasugi et al., 1983; Ow et al., 1994). The $(\gamma EC)_nG\text{-Cd-}S^{2-}$ complex has a greater stability and higher Cd-binding capacity than the low-molecular-weight complex, and has a structure consisting of a CdS crystallite core and an outer layer of $(\gamma EC)_nG$ peptides (Dameron et al., 1989). The higher binding capacity of the sulfide-containing complex confers a greater degree of tolerance to Cd (Ow et al., 1994). In *S. pombe*, evidence has also been presented for subsequent vacuolar localization of $(\gamma EC)_nG\text{-Cd-}S^{2-}$ complexes (Ortiz et al., 1992, 1995; Ow, 1993), confirming a link between cytosolic sequestration and vacuolar compartmentation. Although the main function of *S. cerevisiae* metallothionein (yeast MT) is cellular copper homeostasis, induction and synthesis of MT, as well as amplification of MT genes, lead to enhanced copper resistance in both *S. cerevisiae* and *C. glabrata* (Macreadie et al., 1994; Howe et al., 1997). Production of MT has been detected in both Cu- and Cd-resistant strains of *S. cerevisiae* (Tohoyama et al., 1995; Inouhe et al., 1996). However, it should be noted that other determinants of tolerance also occur in these and other organisms, e.g., transport phenomena (Gadd and White, 1989; Inouhe et al., 1996; Yu et al., 1996), while some organisms, e.g. *Kluyveromyces lactis*, are not capable of MT or $(\gamma EC)_nG$ synthesis (Macreadie et al., 1994). In *S. cerevisiae*, it has been shown that changes in amino acid pools can occur in response to nickel exposure with the formation of vacuolar nickel–histidine complexes proposed as a survival mechanism (Joho et al., 1995). Little work has been carried out on MT or $(\gamma EC)_nG$ peptides in filamentous fungi to date (see Gadd, 1993a; Galli et al., 1994; Howe et al., 1997; Kameo et al., 2000).

37.6 METAL TRANSFORMATIONS

The mechanisms by which fungi (and other microorganisms) effect changes in metal speciation and mobility are important components of biogeochemical cycles for metals, as well as all other elements, including carbon, nitrogen, sulfur, and phosphorus, with additional implications for plant productivity and human health (Leyval et al., 1993; Gadd, 1999). Mineral components of soil contain considerable quantities of metals that are biologically unavailable. Certain microbial processes dissolve metal minerals, thereby increasing metal bioavailability and potential toxicity, whereas others immobilize them and reduce bioavailability. However, it should be realized that there is not necessarily any direct relationship between bioavailability and toxicity: this is especially true for fungi where acidic pH values can increase chemical availability of metals but greatly reduce toxicity (Gadd, 1992a, 1993a). The relative balance between mobilization and immobilization varies depending on the organisms involved and the physicochemical attributes of their environment. As well as being an integral component of biogeochemical cycles for metals and associated elements, these processes may have potential for the treatment of contaminated solid and liquid wastes.

37.6.1 Metal Mobilization

37.6.1.1 Mechanisms

Metal mobilization by fungi can be achieved by chelation by metabolites and siderophores, and methylation, which can result in volatilization. The biochemical action of fungi on

minerals is believed to be a more important process than mechanical deterioration (penetration and burrowing into otherwise intact mineral material by fungal hyphae) (Kumar and Kumar, 1999). Fungi can solubilize minerals and metal compounds through acidolysis, complexolysis, redoxolysis, and metal accumulation (Burgstaller and Schinner, 1993). So-called heterotrophic leaching by fungi primarily involves the first two mechanisms and occurs as a result of several processes, including proton efflux via the plasma membrane H^+-ATPase, maintenance of charge balance, production of siderophores, or respiratory carbon dioxide accumulation. In many fungal strains, however, heterotrophic leaching occurs mainly through the production of organic acids (e.g., oxalic and citric acid) (Adams et al., 1992; Gadd, 1999, 2000). In addition, other metabolites with metal-complexing properties, e.g., amino acids and phenolic compounds, may also be excreted (Manley and Evans, 1986; Muller et al., 1995). Fungal-derived carboxylic acids play an integral role in chemical attack of mineral surfaces (Muller et al., 1995; Gharieb and Gadd, 1999) and supply both protons and metal-complexing anions (Burgstaller and Schinner, 1993; Gadd, 1999; Gadd and Sayer, 2000). For example, citrate and oxalate can form stable complexes with a large number of metals. Many metal citrates are highly mobile and not readily degraded (Francis et al., 1992). Oxalic acid can also act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe (Strasser et al., 1994). Metal complexation is often dependent on the concentration of anions and metals in solution, pH, and the stability constants of the various complexes (Devevre et al., 1996). Organic acid production is therefore an important agent of mineral deterioration, playing a role in biogenic chemical weathering and soil formation (Gadd, 1999). Solubilization phenomena can also have consequences for mobilization of metals from toxic metal–containing minerals, e.g., pyromorphite ($Pb_5(PO_4)_3Cl$), which can form in urban and industrially contaminated soils. Pyromorphite can be solubilized by phosphate-solubilizing fungi, with concomitant production of lead oxalate (Sayer et al., 1999). Fungi, when exposed to media of reduced iron content, produce Fe(III)-binding ligands, commonly of a hydroxamate nature termed siderophores. These may also act to mobilize other metals, albeit with lower specificity, although the significance of this in the soil has not received detailed study. A variety of fungi can effect methylation of metalloids, e.g., Se, Te, As, to yield volatile derivatives (Gadd, 1993b, 2001b). Microorganisms can also mobilize metals, metalloids, and organometallic compounds and attack mineral surfaces by redox processes (Timonin et al., 1972; Grote and Krumbein, 1992; Gadd, 1993a; De la Torre and Gomez-Alarcon, 1994; Gharieb et al., 1999): Fe(III) and Mn(IV) solubility is increased by reduction to Fe(II) and Mn(II) respectively.

37.6.2 Metal Immobilization

37.6.2.1 Biosorption and Bioaccumulation

Fungal biomass provides a metal sink, either by (1) metal biosorption to biomass (cell walls, pigments, and extracellular polysaccharides); (2) intracellular accumulation and sequestration; or (3) precipitation of metal compounds onto or around hyphae. In addition to immobilizing metals, this also reduces the external free metal activity and may shift the equilibrium to release more metal into the soil solution (Gadd, 1993a, 2000; Sterflinger, 2000). Fungi are highly effective biosorbents for a variety of metals, including Ni, Zn, Ag, Cu, Cd, and Pb (Gadd, 1990, 1993a). Binding of metal ions onto cell walls and other external surfaces can be an important passive process in both living and dead fungal biomass (Gadd, 1990, 1993a; Sterflinger, 2000). Metal-binding capacity can be influenced by environmental pH, with the binding capacity of biomass decreasing at low pH for metals such as Cu, Zn, and Cd (De Rome and Gadd, 1987). Cell density also affects

binding capacity, with lower cell densities allowing a higher yield per unit of biomass (Gadd, 1993a). The presence of melanin and chitin in fungal cell walls also strongly influences the ability of fungi to act as biosorbents (Gadd and Mowll, 1985; Manoli et al., 1997; Fomina and Gadd, 2002). Gadd and Mowll (1985) showed that melanin-containing chlamydospores of *Aureobasidium pullulans* could adsorb three times more Cu than hyaline cells. Manoli et al. (1997) demonstrated that chitin, a nitrogen-containing polysaccharide and major component of fungal cell walls, is a substrate on which calcite will readily nucleate and subsequently grow, favoring deposition from supersaturated solution at pH 8.5 at 25°C.

37.6.2.2 Formation of Biogenic Minerals (Mycogenic Precipitates) by Fungi

Fungi have been shown to precipitate a number of inorganic and organic compounds, e.g., oxalates and oxides (Arnott, 1995; Verrecchia, 2000; Gadd, 2000; Grote and Krumbein, 1992) (Figure 37.1d to f). Precipitation, including crystallization, will immobilize metals in the soil environment and therefore limit bioavailability, as well as lead to release of nutrients like sulfate and phosphate (Gadd, 2000). Calcium oxalate crystals are commonly found associated with free-living, pathogenic, and plant symbiotic fungi and are formed by the reprecipitation of solubilized calcium as calcium oxalate (Arnott, 1995). The formation of calcium oxalate by fungi has a profound effect on biological and geochemical processes in soils, acting as a reservoir for calcium in the ecosystem, but also influencing phosphate availability (Gadd, 1993a, 1999, 2000). Fungi can also produce other metal oxalates with a variety of different metals and metal-bearing minerals, e.g., Cd, Co, Cu, Mn, Sr, Zn, and Ni (Gadd, 2000; Magyarosy et al., 2002) (Figure 37.5b and c). The formation of metal oxalates may provide a mechanism whereby fungi can tolerate environments containing potentially high concentrations of toxic metals. A similar mechanism is thought to occur in lichens observed growing on copper sulfide–bearing rocks, where precipitation of copper oxalate occurs within the thallus (Arnott, 1995; Easton, 1997). Under laboratory conditions, media amended with powdered minerals have revealed fungal production of metal oxalates, which adhere to fungal hyphae or are deposited nearby (Sayer et al., 1997) (Figure 37.5b and c). However, oxalates are not the only crystalline precipitates to be associated with fungal hyphae and lichen thalli. The precipitation of carbonates on fungal hyphae, particularly calcite ($CaCO_3$), has been reported in calcareous soils and near surface limestones (calcretes) (Kahle, 1977; Klappa, 1979; Calvet, 1982; Callot et al., 1985a, 1985b; Verrecchia et al., 1990; Monger and Adams, 1996; Bruand and Duval, 1999). Desert varnish, an oxidized metal layer (patina) a few millimeters thick found on rocks and in soils of arid and semiarid regions, is believed to be of fungal and bacterial origin. For example, fungi of the *Lichenothelia* genus can oxidize manganese and iron in metal-bearing minerals such as siderite ($FeCO_3$) and rhodochrosite ($MnCO_3$), or from metals absorbed from rainfall or windblown dust, and precipitate them as oxides (Grote and Krumbein, 1992).

37.7 SIGNIFICANCE OF FUNGI IN GEOCHEMICAL CYCLES OF METALS: PERSPECTIVES FOR BIOREMEDIATION

Toxic metals in natural, industrial, and agricultural soils are a risk to human health. Some of the processes outlined above have the potential for treatment of contaminated land (Gadd, 2000; Hochella, 2002). Solubilization processes provide a route for removal of metals from soil matrices, whereas immobilization processes enable metals to be trans-

formed into insoluble chemically more inert forms. These processes could be used *in situ*, but are possibly best suited for use in bioreactors (Gadd, 2000). Living or dead fungal biomass and fungal metabolites have been used to remove metal or metalloid species, compounds and particulates, and organometal(loid) compounds from solution. There has also been the use of extracellular ligands excreted by fungi, especially from *Aspergillus* and *Penicillium* spp., to leach metals such as Zn, Cu, Ni, and Co from a variety of solid materials, including low-grade mineral ores (Brandl, 2001).

Mycorrhizal associations could also be used for metal cleanup. Phytoextraction involves the use of plants to remove toxic metals from soil by accumulation in aboveground parts. Mycorrhizas may enhance phytoextraction directly or indirectly by increasing plant biomass, and some studies have shown increased plant accumulation of metals when inoculated with mycorrhizal fungi isolated from metalliferous environments. Plant symbiotic mycorrhizal fungi can accumulate metals from soil components, and this may have consequences for both essential metal nutrition of the symbiosis and increased or decreased toxicity. Because plants growing on metalliferous soils are generally mycorrhizal, an important ecological role for the fungus has frequently been postulated, although such a role, e.g., phytoprotection, is often difficult to establish. Mycorrhizal fungi exhibit "constitutive and adaptive resistance" to metals (Meharg and Cairney, 2000), although the relative contributions of passive and active processes in overall response are seldom elucidated (Gadd, 1993a). Arbuscular mycorrhizas from metal-contaminated sites are often more metal tolerant to, e.g., Cd and Zn, than other isolates, suggesting a possible benefit to the plant via increased metal tolerance, nutrient uptake, etc., though in some instances, AM plants do not necessarily require fungal colonization for survival (Griffioen, 1994). For ECM fungi, it appears that these organisms possess constitutive as well as adaptive properties of resistance (Colpaert and Van Assche, 1987, 1992, 1993; C. Hartley et al., 1997). It is often postulated that mycorrhizas provide a barrier to the uptake of potentially toxic metals (Wilkins, 1991; Hetrick et al., 1994; Wilkinson and Dickinson, 1995; Leyval et al., 1997; Meharg and Cairney, 2000) though this has not been confirmed in every case. Further, in some instances, AM may mediate enhanced accumulation of essential metals, which unless regulated may lead to phytotoxicity (Killham and Firestone, 1983). It is generally concluded that local conditions in metal-contaminated sites may determine the cost–benefit relationship between the plant and the AM fungus, since detrimental, neutral, or beneficial interactions have all been documented (Meharg and Cairney, 2000). For ericoid mycorrhizas, clear host protection is observed for ericaceous plants, e.g., *Calluna* sp., *Erica* sp., and *Vaccinium* sp. growing on Cu- and Zn-polluted or naturally metalliferous soils, the fungus preventing metal translocation to aerial plant shoots (Bradley et al., 1981, 1982). Further, ericaceous plants are generally found on nutrient-deficient soils, and it is likely the mycorrhiza could additionally benefit the plants by enhanced nutrient uptake (Smith and Read, 1997). A protective metal-binding effect of ECM fungi has been postulated frequently (e.g., Leyval et al., 1997), though other workers point out the lack of clear evidence for this (Dixon and Buschena, 1988; Colpaert and Van Assche, 1993). However, ECM plants possessed higher tissue P concentrations, indicating some benefit from the association (Meharg and Cairney, 2000).

37.8 CONCLUDING REMARKS

- Toxic metal contamination of soils leads to a decrease in microbial diversity and may result in a shift in the microbial population from bacteria to fungi, and

shifts within fungal communities as a result of the selection of metal-tolerant species and strains, and the possible induction of resistance mechanisms within fungal species.

- Toxic metals can inhibit growth and spore germination of fungi, affect reproduction and metabolic activity, and reduce the ability of mycorrhizal fungi to colonize host plants. Metal toxicity may depend on the concentrations of available nutrients and other physicochemical factors such as pH and the degree of sorption to organic and inorganic soil components.
- Toxic metals can cause morphological changes in fungi. In response to toxic metal stress, fungi can adopt various growth strategies, including negative chemotropism, growth cessation, guerrilla and phalanx growth forms, and the formation of cords and synnema, all of which may provide survival advantages
- Mechanisms of metal tolerance and resistance of fungi include reduction of metal uptake and increased metal efflux; metal immobilization outside the biomass, e.g., cell wall sorption; extracellular precipitation of secondary minerals, e.g., oxalates; extracellular metal sequestration by polysaccharides and extracellular metabolites; intracellular chelation, e.g., metallothioneins and phytochelatins; metal localization; and sequestration within vacuoles.
- Fungi can change metal speciation and mobility and are important components of biogeochemical cycles for metals. Fungi can solubilize minerals and metal compounds through acidolysis, complexolysis, redoxolysis, methylation, and the biomass functioning as a metal sink. Immobilization of metals can occur by sorption to biomass (cell walls, pigments, and extracellular polysaccharides), intracellular accumulation and sequestration, precipitation of metal compounds, or neoformation of biogenic minerals, e.g., oxalates.
- The ability of many fungi to withstand toxic metal stress and transform toxic metal species has the potential for treatment of contaminated land and liquid wastes, e.g., removal of metal or metalloid species, compounds and particulates, and organometal(loid) compounds from solution by biosorption, and metal leaching of solid materials and wastes, including low-grade mineral ores. Metal-tolerant mycorrhizal associations could also be used in phytoremediation and restoration/reforestation of contaminated land.

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38

Radionuclides and Fungal Communities

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38.1 INTRODUCTION

Fungi have been shown to take up and translocate in their mycelium a vast array of naturally occurring as well as man-made radionuclides. Among the latter, in most cases radiocesium is prominent. Under pure culture conditions, influx of ^{137}Cs into fungal hyphae ranges from 44 (*Fusarium* sp.) to 276 (*Mycena polygramma*) nmol Cs g^{-1} dry weight h^{-1} (Clint et al., 1991; Dighton et al., 1991). In nature, the species-specific accumulation of ^{137}Cs in basidiocarps was already described before Chernobyl (see, e.g., Haselwandter, 1978). Hence, after Chernobyl a selection of species analyzed earlier could be used for biomonitoring the radioactive contamination of the environment (cf. Haselwandter et al., 1988; Grodzinskaya et al., 1995). Data on ^{137}Cs contamination of fruit bodies of selected species of basidiomycetes were compiled by Haselwandter and Berreck (1994). Since that review, further analytical data have been published in agreement with observations made earlier.

It was demonstrated again that basidiocarps of the genus *Cortinarius* accumulate especially high levels of ^{137}Cs (Rafferty et al., 1999). In most fungal samples activity concentrations of radiocesium are higher than for ^{210}Pb and ^{226}Ra (Kirchner and Daillant, 1998). Radiocesium activity concentrations in the fruit bodies of macrofungi are generally higher than in many other foodstuffs (Barnett et al., 1999). Consumption of mushrooms by rural populations in Russia (Bryansk region) are considered to be the main reason for a 60 to 70% mean increase in radiocesium activity concentrations in humans in autumn (Skuterud et al., 1997). In a coastal pine forest in Japan, both ^{137}Cs and stable Cs concen-

trations, as well as the specific transfer factors, are higher in mushrooms than in common agricultural plants; for ^{137}Cs, the transfer factors are equivalent to those for ^{134}Cs and significantly correlated to those of stable Cs (Tsukada et al., 1998). Also in the Kola Peninsula the activity concentrations of ^{137}Cs in fungi, lichens, and mosses are significantly higher than in grasses and potatoes and two orders of magnitude higher in reindeer meat than in locally produced beef and pork (Travnikova et al., 2002); in case of reindeer breeders, consumption of reindeer meat, mushrooms, and other radioactive contaminated foodstuffs is made responsible for elevated committed doses in the range of 10 to 15 μSv per month.

38.2 FUNGAL ROLES IN CYCLING OF RADIONUCLIDES

Within the soil microbiota, the mycobiota play a key role in the mobilization and immobilization of radionuclides in the environment, e.g., in forest ecosystems (Steiner et al., 2002). With the enormous biomass fungal mycelia can produce in soils, Cs-accumulating species can substantially contribute to the long-term retention of radiocesium in organic horizons. As shown previously (Berreck and Haselwandter, 1989), the fungal mycelium of a hyperaccumulating species such as *Rozites caperatus* appears to have the potential of taking up radiocesium from deep soil layers and, by doing so, pumping it to top soil layers, which can be anticipated as leading to a recirculation of the radionuclide in upper soil layers and preventing it from moving faster down the soil profile. In addition, this mechanism must be assumed to ensure that basidiomata of hyperaccumulators such as *R. caperatus* and *Cortinarius armillatus* will show remarkably high levels of radiocesium contamination for a long time (Amundsen et al., 1996). Hence, depending on the species composition of fungal mycelia in soil, the fungal biomass must be considered to represent a very important component of the soil determining the time course of the bioavailability of radionuclides, such as those of Cs, and their migration rates in soil. Fruit body production might lead to an important fungal redistribution of ^{137}Cs in the forest floor (Nikolova et al., 1997); based on identification by the restriction fragment length polymorphism (RFLP) pattern, the fungal mantle of ectomycorrhizae and corresponding fruit bodies of *Suillus variegatus* showed about the same ^{137}Cs activity concentrations. Using interaction matrices, a systematic method was applied for development of a conceptual model for the migration of ^{137}Cs in forest ecosystems, and the usefulness of the method was illustrated by a pathway analysis of ^{137}Cs accumulation by fungi (Avila and Moberg, 1999). Based on the use of a three-dimensional stochastic model of radionuclide behavior in forests, it is assumed that the driving force behind the uptake of ^{137}Cs by vegetation is the demand of the plant for potassium (Berg and Shuman, 1995). There are indications that radiocesium continues to be recirculated in biological systems for many years following a pulse of contamination (Avery, 1996).

38.3 HOT PARTICLE DESTRUCTION BY SOIL MICROMYCETES

Fungi isolated from the area of the Chernobyl Nuclear Power Plant (CNPP) frequently show positive radiotropism; i.e., growth of hyphal tips is directed toward a source of low gamma-irradiation (Zhdanova et al., 1994a). Both beta and gamma radiation appear to direct growth of hyphae toward the source of ionizing radiation (Zhdanova et al., 2004). Different strains of *Cladosporium cladosporioides* differ in spore germination, formation

of germ tubes, and hyphal elongation in response to low levels of gamma-irradiation (Zhdanova et al., 2001a). In this fungal species the radiostimulation of conidial germination is not connected to the melanization, but is correlated with the level of radioactivity in the original environment from which the strain is isolated, and its radiotropic capacity. Micromycetes such as *C. cladosporioides* and *Penicillium roseo-purpureum* are able to colonize hot particles with a radioactivity in the range of 330.67 to 31.45×10^3 (*P. roseo-purpureum)* to 5.55×10^2 to 4.01×10^3 Bq (*C. cladosporioides)* and destroy them within ca. 5 months (Zhdanova et al., 1991). Such destruction leads to a reduction in particle size, which can have significant implications for the mobility of radioactive fallout in the form of hot particles in the environment. The ability of some micromycetes to destroy hot particles, thus making radionuclides more easily accessible for plant uptake, is assumed to be especially important in regions where most of the radioactive contamination derives from hot particles (Zhdanova et al., 2003).

38.4 RADIOACTIVITY LEADING TO CHANGES IN FUNGAL COMMUNITIES

Before the accident of Chernobyl, soils of different ecosystems in the Kiev region were dominated by nonmelanized fungal genera (Kirilenko, 1978). Based on isolation and identification of fungi employing standard techniques, melanin-containing fungi appear to be dominant with increasing radionuclide pollution, forming complex communities mainly consisting of fungal genera such as *Stachybotrys*, *Ulocladium*, *Preussia*, *Plenodomus*, *Humicola*, *Aureobasidium*, and *Alternaria* (Zhdanova et al., 1994b). Soils containing moderate levels of radioactive pollution seem to contain a higher proportion of nonmelanized genera. A study carried out between 1987 and 1989 on the basis of a filter technique revealed greater lengths of dark-pigmented mycelium at the beginning of the survey, which then changed to a predominance of light-colored mycelium toward the end of the study (Vasilevskaya et al., 1993). It was assumed that melanized fungi are more resistant to ionizing radiation than nonmelanized forms (Zhdanova and Vasilevskaya, 1988). However, filamentous fungi show intraspecific variation in their gamma-radiation resistance. Strains from *Alternaria alternata* vary from supersensitive to highly resistant, with nearly all strains originating from the highly radiation polluted reactor block 4 of the Chernobyl Nuclear Power Plant, possessing high radiation resistance and a well-conserved genome structure (Mironenko et al., 2000). As in soil, CNPP block 4 studies of fungal biodiversity and prevalence coefficients revealed a dominance of melanin-containing species in this heavily contaminated habitat (up to 220 mR h^{-1}) (Zhdanova et al., 2000). Table 38.1 provides further evidence for the predominance of melanized fungal species in locations of block 4 with even higher radioactivity (500 to 2500 mR h^{-1}).

It would be of interest to know whether the predominance of melanized genera in soil is reflected by a similar prevalence in the atmosphere of spores derived from such fungi. Albeit growing season and altitude dependent, spores of melanized fungi were shown present together with nonmelanized forms, in their contribution to the allergenic components of the air (Ebner et al., 1989, 1992). Furthermore, plant roots are most commonly infected by not only mycorrhizal fungi, but also dark septate root endophytes (Haselwandter, 1997). In that respect, it would be of interest to know whether a strong contamination of the environment with radionuclides leads to a shift in infection intensities caused by dark septate root endophytes, especially of the *Phialocepala* type.

Table 38.1 Micromycetes Isolated from Locations in the Fourth Unit of the Chernobyl Nuclear Power Plant with High Levels of Radioactivity (500 mR h^{-1} and more)

Species	Frequency %	Melanization	Origin	Level of Radioactivity mR h^{-1}
Aspergillus versicolor	5.55	–	Site 009/4, leaden coating of floor	1000
Aureobasidium pullulans	5.55	+	Undercoating space, beam, axle L-K, row 46	2500
Alternaria alternata	5.55	+	Light coating of shelter, axle L, row 45	2500
Botrytis cinerea	5.55	+	Site 009/4, leaden coating of floor	1000
Cladosporium cladosporioides	5.55	+	Exit to roof, concrete wall over the door	800
C. herbarum	11.11	+	Undercoating space, beam, axle L-K, row 46; light coating of shelter, axle L, row 45	2500
Cladosporium sp.	5.55	+	Light coating of shelter, axle L, row 45	2500
Dematiaceous fungi	5.55	+	Undercoating space, beam, axle L-K, row 46	2500
Hyalodendron sp.	5.55	–	Light coating of shelter, axle L 45, row 45	2500
Mycelia sterilia (dark)	16.67	+	Sites 211/2 and 009/4, leaden coating of floor	500, 1000
M. sterilia (orange)	5.55	–	Undercoating space, beam, axle L-K, row 46	2500
Penicillium sp.	11.11	–	Undercoating space, beam, axle L-K, row 46; site 009/4, leaden coating of floor	2500, 1000
P. funiculosum	5.55	–	Site 009/4, leaden coating of floor	1000
P. olsoni	5.55	–	Site 009/4, tank (cistern) surface	1000

Note: Indicated frequency (%) of discovery of fungal species, i.e., number of isolates allocated to single species or fungal entity divided by total number of fungal isolates (n = 36) obtained from a total of 14 samples investigated, and multiplied by a factor of 100. Sampling procedures and isolation of fungi as in Zhdanova et al. (2000). Origin indicates source of reference strain deposited in culture collection of the Institute of Microbiology and Virology, National Academy of Sciences of the Ukraine, Kiev.

38.5 FUNGI AS BIOINDICATORS FOR RADIOACTIVE CONTAMINATION OF THE ENVIRONMENT

The potential of fungi to accumulate radionuclides in a species-specific manner can be used to monitor radioactivity in the environment (Haselwandter and Berreck, 1994). In 1986, after the Chernobyl accident, the ^{137}Cs content of basidiocarps of a set of seven species was 3.0 to 4.8 times higher than in 1974 (Haselwandter et al., 1988). A close correlation between radiocesium content of fungal fruit bodies and the deposition of radiocesium in the sampling area was demonstrated, e.g., for Finland (Berreck et al., 1992). A study carried out in the Ukraine over 6 years (1993 to 1999) confirmed that basidiocarps, especially of hyperaccumulating species, could be used for long-term radioecological monitoring of areas contaminated with radiocesium, for instance, as a result of the Chernobyl accident (Grodzinskaya et al., 2003).

As the pattern of accumulation of both radionuclides deriving from Chernobyl and trace elements appears to be site specific, such data were discussed as useful tools for the identification of the origin of basidiocarps (Marzano et al., 2001). In areas where fruit bodies of species such as *Boletus edulis* and *Boletus aestivalis* achieve a high commercial value, this approach was claimed to be useful for controlling the correct indication of the provenance of fungi sold on the market.

In addition to macromycetes, soil micromycetes can also be used for assessing the radioactive contamination of the environment. It was proposed that the frequency with which species such as *Chaetomium aureum* and *Paecilomyces lilacinus* can be isolated from soil is indicative of a high level of radioactive pollution (3.7×10^5 to 3.7×10^7 Bq kg^{-1} soil), whereas *Acremonium strictum* and *Metarrhizium anisopliae* are typical for an intermediate pollution level (3.7×10 to 3.7×10^5 Bq kg^{-1} soil), and *Myrothecium roridum*, *M. verrucaria*, and *Arthrinium phaeospermum* indicate low contamination (3.7 to 3.7×10 Bq kg^{-1} soil) (Zhdanova et al., 1995, 2001b). On that basis, the rehabilitation process could be monitored following radiocesium contamination of the soil. Observation of a shift in community structure with special emphasis on the species listed above could possibly allow for an evaluation of the effect of countermeasures taken after radioactive contamination of the environment.

38.6 RADIONUCLIDE TRANSFER FROM SOIL INTO PLANTS AS AFFECTED BY MYCORRHIZAL FUNGI

Depending on the specific type of mycorrhiza formed by a given plant, the soil–plant transfer of heavy metals, including radionuclides, appears to be mediated by arbuscular, ericoid, or ectomycorrhizal fungi (Haselwandter et al., 1994; Leyval et al., 1997; Drissner et al., 1998; Strandberg and Johansson, 1998). Formation of ectomycorrhizae with *Hebeloma crustuliniforme* led to a 21 to 27% reduction in cesium uptake by *Picea abies* seedlings when the substrate concentration of Cs was equal to or higher than that of K (Brunner et al., 1996). In case of *Calluna vulgaris*, formation of ericoid mycorrhizae in most treatments resulted in a significantly higher transfer of ^{134}Cs to the shoots than in nonmycorrhizal heather (Strandberg and Johansson, 1998); however, application of K at a rate of 10 kg ha^{-1} reduced the ^{134}Cs concentration in the shoots of *Calluna* by 49% as an average. In *Agrostis tenuis*, arbuscular mycorrhizal colonization by *Glomus mosseae* led to a significant decrease in shoot Cs content of the grass from the first (4 weeks) to the third (8 weeks) harvest (Berreck and Haselwandter, 2001); addition of K equivalent to 100 kg ha^{-1} produced a significant decrease in Cs uptake by both arbuscular mycorrhizal

and nonmycorrhizal plants over a growing period of 10 weeks. On the other hand, in each of three other grass species (*Paspalum notatum*, *Sorghum halpense*, *Panicum virginatum*) arbuscular mycorrhiza formation with *G. mosseae* or *Glomus intraradices* enhanced the concentrations of ^{137}Cs and ^{90}Sr over the uninoculated controls at each harvest (Entry et al., 1999). Under root-organ culture conditions, the extraradical mycelium of *Glomus lamellosum* appears to take up and possibly accumulate ^{137}Cs (Declerck et al., 2003). Also under root-organ culture conditions, the uranium flux rate was claimed to be higher in extraradical hyphae of the arbuscular mycorrhizal fungus *G. intraradices* than in carrot roots, while the intraradical hyphae seemed to significantly contribute to the U immobilization by mycorrhizal roots (Rufyikiri et al., 2003). However, the validity of data on translocation measured under such experimental conditions is bound to be restricted because a root-organ culture does not allow, e.g., for the establishment of proper source–sink relationships, which in nature are of paramount importance for translocation of cations. Nevertheless, the examples selected from current literature illustrate that mycorrhizal infection plays a key role with regard to radionuclide uptake by plants and can lead to either a decrease or an increase in radionuclide concentration of plant tissues. However, under some experimental conditions, arbuscular mycorrhizal fungi may have no effect on the radiocesium transfer from soil to plants. This was demonstrated by Jones et al. (2004) on the basis of experiments in which the plants were supplied with a surplus of K. Hence, the results of these experiments are not surprising; they even corroborate the well-known recommendation of K fertilization as a countermeasure to Cs uptake by plants. While other controls have been included in the experimental design, the control of utmost importance with regard to the objective of this study is missing, i.e., the same set of experiments under low K conditions.

38.7 CONCLUDING REMARKS AND OUTLOOK

Bioremediation is considered to be a cost-effective technology for the treatment of sites contaminated with a variety of pollutants (Atlas and Unterman, 1999). Fungi, in particular, can be exploited as bioremediation agents in metal-contaminated soil (Gray, 1998). Following from statements made above, arbuscular mycorrhizal fungi, for example, have the potential to be used for both soil remediation, on the basis of plants accumulating large amounts of radiocesium, and the prevention of Cs from entry into the food chain (Leyval et al., 2002). Based on the use of a nonmycorrhizal plant for phytoextraction, Ebbs et al. (2000) have estimated the achievement of a 75% decrease of ^{137}Cs contamination in about 15 years. However, it would appear essential to compare such potential with that of mycorrhizal plant species, which could also be used for phytoextraction of radiocesium, as suggested, for example, by Entry et al. (1999). After contamination of the biosphere with radionuclides, an alternative strategy aims at preventing radiocesium from entering the food chain. As outlined above, in that respect arbuscular mycorrhizal fungi can also play a key role through reducing the Cs uptake by plants from soil (Berreck and Haselwandter, 2001).

It is of interest to note that the biological function of fungi in the environment appears to be even more important with increasing radioactivity levels. Such an increase seems to lead to a predominance of dark-pigmented fungal mycelia, possibly with a higher radionuclide adsorption capacity than nonmelanized forms. The fungal community structure appears to shift toward species that may be more radiation resistant. Furthermore, radiotropism together with hot particle destruction must be anticipated to have radioecological consequences as outlined above. However, a thorough understanding of the mechanisms underlying these observations is still lacking, but would be most desirable.

It must be emphasized that so far, the information on radionuclides and fungi that is available in the literature and summarized here focuses mainly on macrofungi or culturable microfungi, including arbuscular mycorrhizal fungi that can be grown in co-culture with suitable host plants. That means the vast majority of fungi still represent a huge black box, the importance of which, with regard to radionuclide interactions, we are unable to estimate, at least at present. Hence, it would be of particular value to study the role of nonculturable fungi vs. culturable species in that respect. Hopefully, the recent developments in the molecular ecology of fungi will provide important insights for a deeper understanding of the radionuclide–fungus interaction in nature. Information of this kind could then be used for the development of novel biotechnological approaches, e.g., for bioremediation.

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39

How Do Composition, Structure, and Function of Mycorrhizal Fungal Communities Respond to Nitrogen Deposition and Ozone Exposure?

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39.1 INTRODUCTION

This review will focus specifically on how mycorrhizal fungal (MF) communities are structured by, and function under, air pollution. In order to understand the impacts of air pollution on MF communities, and the functional significance of those impacts, we must consider both the empirical evidence for air pollution effects on those communities and the mechanisms by which fungal communities are likely to be affected. By understanding these mechanisms, we should also gain some insight into the potential functional consequences of community change.

Human effects on atmospheric chemistry have been dramatic, including increases in chemical oxidants (e.g., SO_2, NO_2, O_3), nutrient and acidifying compounds (e.g., SO_4^{2-}, NO_3^-, NH_y), and greenhouse gases (e.g., CO_2, CH_4, N_2O) driven largely by increased industrial and agricultural activity (Schlesinger, 1997). For example, anthropogenic N fixation has led to a doubling of terrestrial N fixation, and greatly elevated levels of N deposition (Galloway and Cowling, 2002); average tropospheric ozone (O_3) levels have increased from preindustrial levels of <20 ppb (Mickley et al., 2001) to current average levels of over 50 ppb, with high variability leading to regional levels of over 100 ppb (Ehhalt et al., 2001); and atmospheric CO_2 has increased over 30% compared with preindustrial levels (Houghton et al., 2001). The increases in these compounds have altered ecosystem structure and function in a variety of ways. Most relevant to the current chapter,

there are indications that diversity and community structure of mycorrhizal fungi have been affected by pollutants. Much less clear is what the exact causal mechanisms and functional consequences of these changes are.

This chapter will focus largely on the effects of atmospheric N and chemical oxidants, especially O_3, on mycorrhizal fungal communities. Along with CO_2, these are the pollutant classes with the most ubiquitous effects on ecosystems. The effect of changes in atmospheric CO_2 concentrations on mycorrhizal fungal communities is addressed elsewhere in this volume (Chapter 36), so we will address CO_2 primarily in terms of possible interaction with reactive N and O_3. We will attempt to answer the following questions: What are the potential pathways of pollutant effects on mycorrhizal fungi? Is there any evidence that mycorrhizal fungal communities are affected by air pollution? What are the potential functional consequences of mycorrhizal fungal community change? Where are our knowledge gaps and how might they be filled? It is hoped that this chapter will complement other excellent reviews of pollutant effects (e.g., Dighton and Jansen, 1991; Wallenda and Kottke, 1998; Erland and Taylor, 2002; Andersen, 2003).

39.2 POTENTIAL PATHWAYS OF POLLUTANT EFFECTS

In order to understand how air pollution might affect mycorrhizal fungi, the first point to consider is how the unique traits of mycorrhizal fungi define pathways of pollutant exposure. One of the properties of filamentous fungi that distinguishes them from most other sessile soil microorganisms is their ability to capture and use spatially discrete resources. The importance of this trait is obvious in the mycorrhizal fungi. By symbiotically bridging the root and soil environments, they are able to gain access to two valuable resource pools: root carbohydrates and soil resources. Although the advantages of this habit are made clear by mycorrhizal fungal diversity (Horton and Bruns, 2001) and dominance in many soils (e.g., Wallander et al., 2001), a disadvantage is that mycorrhizal fungi are vulnerable to environmental factors that alter resources and conditions in the host and soil environment.

Reactive nitrogen (NO_y, NH_x) and acidifying and oxidant air pollution are prime examples of such environmental factors, having the potential to affect mycorrhizal fungi via alteration of both host and soil environments (Figure 39.1). The likely pathway of these effects will differ depending on the mode of pollutant action.

39.2.1 Chemical Oxidants

We will focus on ozone (O_3) as the best-studied oxidant, although SO_2 is another environmentally important oxidant. At sufficiently high levels, O_3 damages leaves, reducing photosynthetic C gain, increasing leaf metabolic costs, and possibly reducing phloem loading (Andersen, 2003 and references therein). As a result, with sufficient doses O_3 often leads to reduced host C flux to roots and mycorrhizae (Andersen, 2003; Figure 39.1). Thus, initial effects on mycorrhizal fungi are likely to be via changes in C availability and perhaps host receptivity, although it is possible that longer-term effects could occur via indirect effects of oxidants on plant nutrition arising from reduced nutrient uptake, or via alteration of soils resulting from changes in quality and quantity of organic matter inputs (Figure 39.1).

39.2.2 N Deposition

In contrast to oxidants, N deposition has the potential to affect mycorrhizal fungi both via alterations in soils and consequent effects on soil resources and conditions experienced by

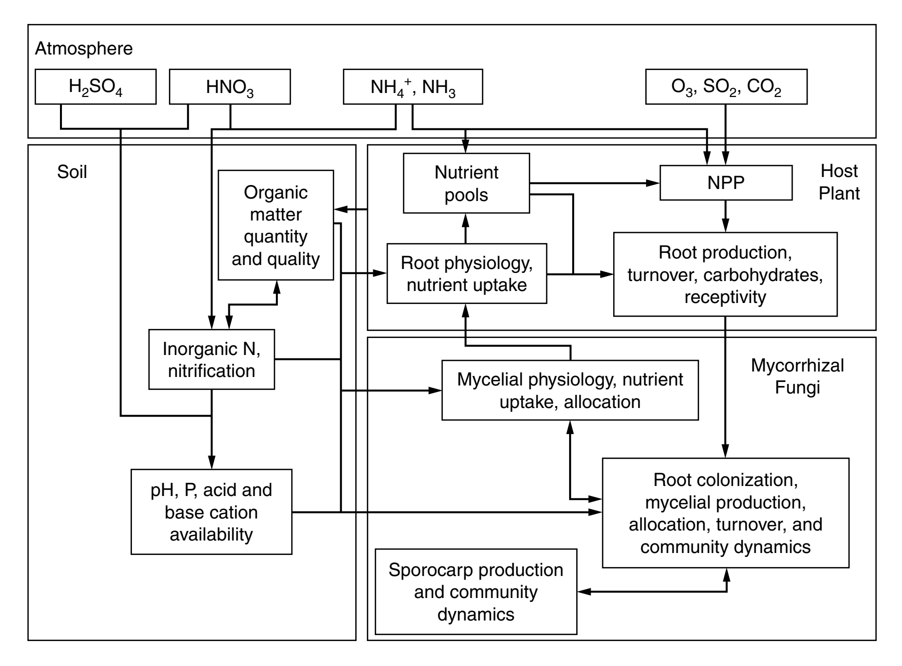


Figure 39.1 Conceptual diagram of the potential pathways of air pollution effects on mycorrhizal fungal communities.

MF (inorganic N availability, soil pH, base cation and P availability, acid cation availability, especially Al^{3+}) (Aber et al., 1998) and via alteration of host plant nutrition and the consequent effects on host C supply to roots and host receptivity to fungi (Figure 39.1).

It is important to know whether pollution-related changes in mycorrhizal fungal communities are driven largely by plant choice of ectomycorrhizal fungi (EMF), by fungal ability to colonize roots, or by a changing abiotic environment, because these mechanisms could lead to very different functional outcomes. Before we address these alternatives, we will consider the empirical evidence for mycorrhizal fungal community change.

39.3 EVIDENCE OF MYCORRHIZAL FUNGAL COMMUNITY CHANGE IN RESPONSE TO AIR POLLUTION

39.3.1 Variety of Study Types and Conditions

There are a range of approaches to investigating pollution effects on mycorrhizal fungi. The range of approaches varies with the type of pollutant. For all pollutants, experimental and gradient approaches have been used. The experimental approaches generally provide a higher degree of control but less realism. The gradient approach varies from large-scale regional gradients that usually are associated with variation not only in pollutants, but also in a range of other environmental variables, to more local gradients associated with point sources of air pollution, where extraneous environmental variables are held relatively constant.

For simulation of nutrient or acidity effects, the experimental approaches involve additions of nutrients in dry or liquid form. The timing of additions varies hugely, with some studies applying nutrients in one pulse in the first year only, one addition per year, or multiple additions per year, with the last most closely mimicking atmospheric deposition. Experimental settings include field plots, greenhouses, and growth chambers.

For oxidants there are also a range of exposure systems, with an evolution from studies carried out in growth chambers or greenhouses, to the field in closed chambers or open-topped chambers, to free-air addition of gases. The degree of realism increases with each of these changes, with parallel increases in cost and decreases in the degree of control.

Experimental outcomes and information derived from studies can be affected by a variety of factors, including host plant species and genotypes, host age/size, fungal inocula, variation in baseline or ambient pollution levels, amount and timing of additions or exposures, length of time of experimental exposures/treatments, soil physical, chemical, and biological properties, method of fungal identification, and a range of other factors. Given all of the methodological variability, it is not surprising that there is also variability in experimental outcomes. In the following, we will attempt to determine whether there is any consistent pattern in mycorrhizal fungal community response arising from these studies.

39.3.2 Field Studies: N Inputs and EMF Sporocarps

For ectomycorrhizal fungi, sporocarps are our most spatially and temporally extensive window into pollution effects on mycorrhizal fungi. This is especially true in Europe, where a long tradition of field mycology has resulted in some excellent long-term records of fungal distributions. It is therefore not surprising that some of the first evidence that ectomycorrhizal fungal communities might be negatively affected by air pollution came from longitudinal studies and spatial patterns of fungal sporocarps in Europe. These studies, summarized in Arnolds (1991), indicated that a broad range of ectomycorrhizal fungal species were decreasing in abundance or disappearing over large regions of Europe exposed to a broad range of pollutants. In contrast, no such declines were seen in saprophytic fungi. This suggested that some unique aspect of the mycorrhizal habit was leading to such declines. One interesting feature of these declines is that certain genera of ectomycorrhizal fungi appeared to be more affected than others (Figure 39.2).

Arnolds (1991) suggested multiple possible causal mechanisms for the decline. These included natural factors, sporocarp collection, forest management, and air pollution. Of these, he considered air pollution to be a likely causal agent. In particular, the patterns of sporocarp decline were most consistent with the effects of nitrogen fertilization. Fertilization experiments and deposition gradient studies demonstrated that additions of N can lead to changes in sporocarp production that paralleled those seen over time in Europe (Wallenda and Kottke, 1998 and references therein; Lilleskov et al., 2001; Peter et al., 2001). A larger-scale complex pollutant gradient study suggested that SO_2 and NH_3 were both negatively correlated with EMF species richness (Termorshuizen and Schaffers, 1987). A more localized N deposition gradient study also found very strong negative relationships between a broad range of N availability measures and sporocarp diversity (Figure 39.3; Lilleskov et al., 2001). Fertilization studies (Menge and Grand, 1978; Wästerlund, 1982; Termorshuizen, 1993; Wiklund et al., 1995; Brandrud and Timmerman, 1998; Peter et al., 2001) also found reduced species diversity and sporocarp production with elevated N inputs. These declines were not equal across taxa, with certain taxa declining in abundance and diversity more rapidly, including *Cortinarius* (Wästerlund, 1982; Termorshuizen, 1993; Brandrud, 1995; Brandrud and Timmermann, 1998; Lilleskov et al., 2001), *Russula* (Brandrud, 1995; Lilleskov et al., 2001), and *Suillus* (Menge and Grand, 1978; Wästerlund, 1982). Several other taxa appear to continue to fruit at higher deposition levels, including *Lactarius rufus* and *Paxillus involutus* (Hora, 1959; Laiho, 1970; Ohenoja, 1978; Salo, 1979; Wästerlund, 1982; Brandrud, 1995), *Lactarius theiogalus* (Brandrud,

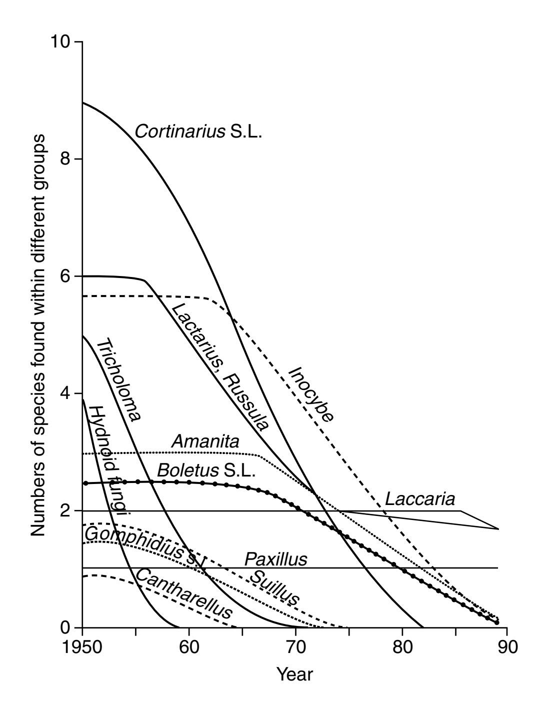


Figure 39.2 Temporal trends in species richness for sporocarp collections from different genera of ectomycorrhizal fungi over time in Europe. (From Arnolds, *Agriculture Ecosystems and Environment*, 35, 209–244, 1991. With permission.)

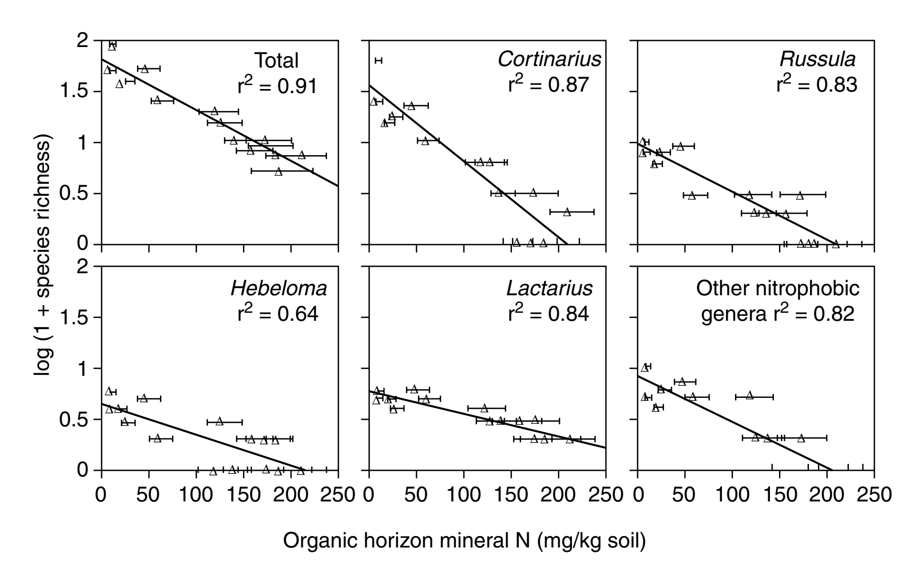


Figure 39.3 Soil inorganic N extractable pools as a predictor of sporocarp species richness for all species, and for the most species-rich genera over a nitrogen deposition gradient in Alaska. (From Lilleskov et al., *Ecological Applications* 11:397–410, 2001. With permission.)

1995; Lilleskov et al., 2001), and *Laccaria* species (Termorshuizen, 1993; Lilleskov et al., 2001).

The exact pattern of response to fertilization seems to vary among studies, with some studies showing declines in fruiting of all taxa, some showing increases in apparently nitrophilic taxa, and others showing initial increases followed by declines. Lilleskov et al. (2001) discuss this pattern in detail, hypothesizing that this variation might be a function of how quickly fertilization shifts forests into a eutrophic state, as a function of the natural N status of the site, land use history, tree species, tree age, amount of N deposited on the site, and size of the initial fertilization.

39.3.3 N Inputs and EMF Communities Belowground

Sporocarp production does not reflect belowground communities directly: first, fungi can reduce allocation to sporocarps in response to N fertilization, without any change in community structure; second, even in the absence of fertilization, ectomycorrhizal fungal species are not equally represented as sporocarps and on roots, presumably because of differences in allocation to sporocarps. Not all ectomycorrhizal fungi produce conspicuous epigeous sporocarps. Some produce thin crust-like sporocarps on logs or leaf litter (e.g., Thelephoraceae and Corticiaceae), and others have no known sexual stage (e.g., *Cenococcum geophilum*). Of those fungi that do produce conspicuous sporocarps, a species' sporocarp production does not necessarily reflect its belowground abundance (e.g., Gardes and Bruns, 1996).

The lack of direct correspondence between communities described via sporocarps and on root tips necessitates the use of belowground studies to characterize the EMF community response. This is more challenging, because identification of fungi on roots is harder, and communities exhibit high diversity and spatial variability. Until the advent of molecular methods, most studies were limited to morphological typing, or morphotyping. This morphotype information is not easily comparable among studies, and morphotyping can lead to false lumping and splitting of taxa (Mehmann et al., 1995). Although some labs have developed morphological typing to a fine art (e.g., Agerer, 1991), currently most studies rely on PCR-based molecular identification methods to obtain reliable community data (Gardes and Bruns, 1996; Horton and Bruns, 2001). These methods are mostly applied to individual root tips, although recently related methods are being applied to fungal hyphae in the soil (Dickie et al., 2002).

Many earlier pot studies of acidic deposition effects on mycorrhizal fungi usually included both HNO_3 and H_2SO_4 in the acidification treatments, although some studies used H_2SO_4 alone. The former were both acidification and fertilization studies. Some studies found no mycorrhizal community response (e.g., Adams and O'Neill, 1991), whereas others found apparent shifts in mycorrhizal community structure. Meier et al. (1989) found that *C. geophilum* increased with decreasing rain pH, an observation anecdotally supported by Stroo and Alexander (1985).

It is worth noting that change in pH in the absence of N addition can apparently alter mycorrhizal fungal communities. For example, Lehto (1994), Dighton and Skeffington (1987), and Erland and Soderstrom (1990) all found that alterations in pH affected the relative proportions of different EMF morphotypes.

Belowground field studies have found that even when sporocarp production responses are rapid and dramatic, short-term mycorrhizal fungal community responses at the root level are minimal. Most short-term studies have shown little or no change belowground (Saunders et al., 1996; Brandrud and Timmermann, 1998; Jonsson et al., 2000; M.F. Allen, personal communication). Two studies (Kårén and Nylund, 1997; Peter et al., 2001) found a belowground response, but it was much less pronounced than the sporocarp

Table 39.1 A Comparison of Response to Atmospheric N Deposition or N Fertilization, and Growth on Protein N in Pure Culture, for Ectomycorrhizal Fungal Taxa for Which Information Is Available

Taxon	Response to N Addition[a]	Reference[b]	Growth on Protein N	Reference[b]
Lactarius theiogalus	+ + +	1, 3	No	9
Paxillus involutus	+ + +	1, 2	Variable	10–13, 18, 20
Lactarius rufus	+ + +	2, 16	Variable	9–12, 18
Laccaria bicolor	+ +[d]	3–5	No–poor	9, 10, 19
Thelephora terrestris	+	1, 2	Variable	10
Tylospora fibrillosa	= / +	1, 2	Variable	13, 18
Cenococcum geophilum	– / =	1, 2	Variable	9, 11, 14, 18
Russula spp.	– / +	3, 6, 7, 15, 17	Yes (1)[c]	18
Cortinarius spp.	– –	1–3, 5	Yes (6)	9, 18
Piloderma croceum group	– –	1, 2	Yes	10, 20
Tricholoma inamoenum	– –	1, 3	Yes	9
Suillus variegatus	– –	2	Yes	10
Suillus bovinus	– –[d]	8	Yes	11, 20

Note: Taxa are ordered by their response to increased N.

[a] For response to fertilization: +, slightly positive; + +, positive; + + +, very positive; =, neutral; –, negative response to fertilization; – –, very negative.

[b] References: 1, Lilleskov et al. (2002a); 2, Kårén (1997); 3, Lilleskov et al. (2001); 4, Ohenoja (1989); 5, Sagara (1992); 6, Brandrud (1995); 7, Shubin et al. (1977); 8, Wästerlund (1982); 9, Lilleskov et al (2002b); 10, Finlay et al. (1992); 11, Abuzinadah and Read (1986); 12, Keller (1996); 13, Ryan and Alexander (1992); 14, El Badaoui and Botton (1989); 15 Avis et al. (2003); 16, Wallenda and Kottke (1998, and references therein); 17, Peter et al. (2001); 18, Taylor et al. (2000); 19, Yamanaka (1999); 20, Bending and Read (1996).

[c] Number in parentheses indicates number of species in genus tested.

[d] Information available from studies of sporocarps only.

response. Whereas for sporocarps there was a significant decline in species richness and diversity, belowground there was no loss of richness or diversity in response to the treatments. However, they did observe shifts in community structure. Kårén and Nylund (1997) found an increase in a brown morphotype that included *Tylospora fibrillosa*, *Lactarius theiogalus*, and other taxa. Peter et al. (2001) found that certain taxa declined in frequency in response to N inputs (e.g., *Russula* spp.), whereas others did not (e.g., *Tylospora asterophora*).

In contrast, studies of the effects of long-term N inputs indicate that long-term effects can be much larger. In both a fertilization study (Kårén, 1997) and an N deposition gradient study (Lilleskov et al., 2002a), high N inputs were associated with strikingly similar changes in belowground ectomycorrhizal fungal communities, resulting in the loss of many ectomycorrhizal fungal taxa and a shift in the dominants (Table 39.1, Figure 39.4). Similarly, along a north–south transect in Europe in which N deposition increased toward the south, Taylor et al. (2000) found a negative relationship between morphotype richness and soil inorganic N in spruce stands and decline in some of the same taxa as in the smaller-scale studies. Although the latter must be viewed with caution

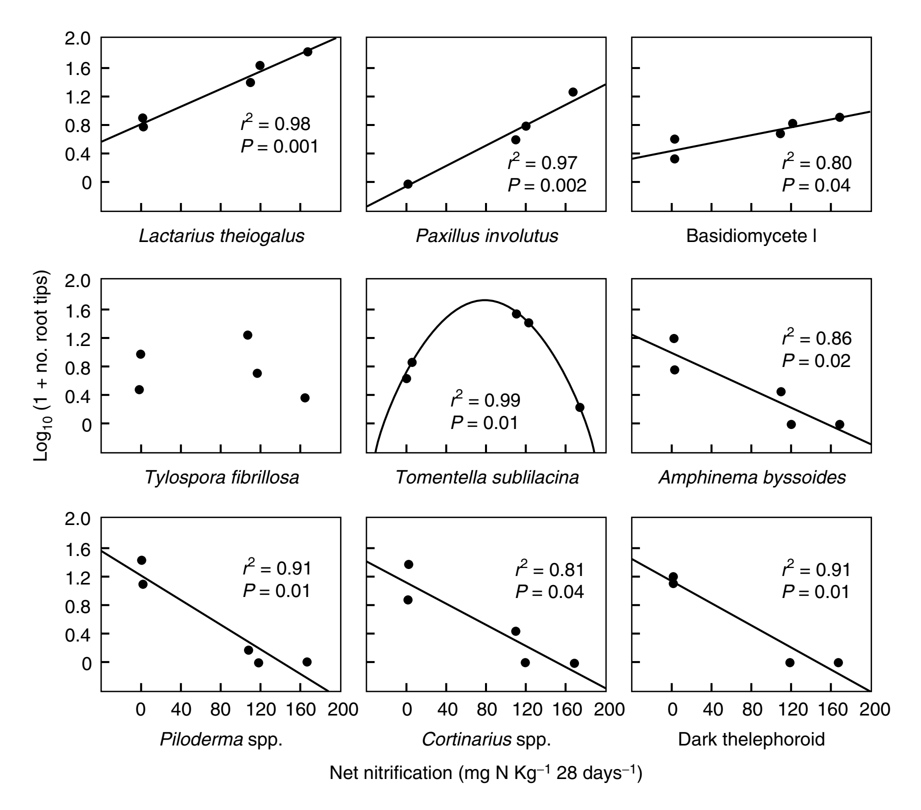


Figure 39.4 Regressions of percentage of white spruce root tips (log transformed) vs. net nitrification for individual ectomycorrhizal fungal taxa over an atmospheric nitrogen deposition gradient near Kenai, AK. (From Lilleskov et al., *Ecology,* 83:104–115, 2002a. With permission.)

given the other parameters varying along the gradient, its consistency with the fertilization and small-scale gradient results lends support to the claim that N deposition is driving belowground EMF community changes across Europe. Taxa that appeared to decline in these studies include *Cortinarius* (Kårén, 1997; Taylor et al., 2000; Lilleskov et al., 2002a), *Piloderma* (Kårén, 1997; Lilleskov et al., 2002a), *Suillus* (Kårén, 1997), many *Tomentella* species (Lilleskov et al., 2002a), *Tricholoma inamoenum*, and *Amphinema byssoides* (Lilleskov et al., 2002a). Taxa that appeared to increase or be relatively unaffected were *Tylospora fibrillosa* (Kårén, 1997; Taylor et al., 2000; Lilleskov et al., 2002a), *Lactarius rufus* (Kårén, 1997), *Lactarius theiogalus*, *Paxillus involutus*, *Tomentella sublilacina*, *Thelephora terrestris*, and an unidentified Corticioid (Lilleskov et al., 2002a). Thus, it appears that lags in the belowground response to N deposition are greater than for the sporocarp response, but long-term inputs are sufficient to cause significant declines in diversity and complete shifts in dominants, at least in boreal conifer forests.

Arnolds (1991) reported that diversity of sporocarps had declined more for conifer-associated than for deciduous-associated ectomycorrhizal fungal taxa. Is this also true belowground? Taylor et al. (2000) also examined beech stands and, in contrast with spruce stands, found a weak positive relationship between morphotype richness and soil inorganic N. In contrast, Avis et al. (2003), examining experimental plots in oak savannah in Minnesota that had been fertilized with complete fertilizer plus moderate or high N for

15 years, found clear declines in EMF sporocarp species richness and shifts in belowground community structure, with declines in *Cortinarius* and increases in *Russula*, but of a smaller magnitude than the shifts found in the long-term conifer studies. The smaller belowground community change compared with long-term conifer fertilization could be due to several factors: different host species, relatively low fertilizer inputs (maximum of 17 kg ha^{-1} $year^{-1}$ above ambient), a base level of complete fertilizer addition that could ameliorate some of the N effects, shorter period (15 vs. >25 years), or other factors such as soil characteristics and climate. These results clearly point to the need for a much more extensive study to determine the generality of mycorrhizal responses to long-term N inputs in different forest types.

The N-induced change in sporocarp production could feed back to affect belowground community composition if certain species require spore inputs to persist in the community. For example, one might expect that species colonizing mature forest stands would be effective at vegetative spread, but it appears that some *Russula* species from mature forests actually have numerous small genets, suggesting either that genets expand very slowly or that continued colonization by spores may be important (Redecker et al., 2001). Given that *Russula* sporocarp production declines with increasing N inputs (Mehmann et al., 1995; Wallenda and Kottke, 1998; Lilleskov et al., 2001; Peter et al., 2001; but see Avis et al., 2003 for an exception), this raises the possibility that reduced spore inoculum may be one mechanism leading to belowground decline of *Russula*. This effect might be weaker in a small-scale fertilization experiment than in a larger-scale regional decline in sporocarp production driven by N deposition because, in the former, local inoculum sources would persist in areas surrounding plots. Thus, small-scale studies might underestimate N deposition effects.

39.3.4 Arbuscular Mycorrhizal Fungi and N Deposition

In contrast with EMF, arbuscular mycorrhizal fungi (AMF) do not produce conspicuous epigeous sporocarps, so there is no long-term record of sporocarp production with which to compare experimental studies. In AMF, much of the information on community composition and structure is from asexual spores collected from the soil. This information has similar constraints to the EMF sporocarp information; i.e., spore abundance does not directly reflect fungal abundance in roots and soil. Molecular identifications, while attainable, are harder to derive from AM roots, because of lower quantities of DNA, and species mixtures in root sections.

There have been a few studies of AMF response to N deposition in coastal sage scrub ecosystems of California, and these suggest that there are significant community changes in AMF communities in response to N inputs. These trends have been indicated by studies of anthropogenic gradients and fertilization experiments (Egerton-Warburton and Allen, 2000) and longitudinal studies of archived soils (Egerton-Warburton et al., 2001). These studies provide multiple lines of evidence that with increasing N availability, there is a corresponding decline in diversity, driven largely by decline or disappearance of the large-spored genera (*Scutellospora*, *Acaulospora*, and *Gigaspora*) compared with smaller-spored *Glomus* species. As in EMF, AMF were more likely to be detected as hyphae in soil than as spores (Egerton-Warburton and Allen, 2000), suggesting that N effects are seen first in spore production for AMF as well.

It is unknown how general this response would be across AMF communities. One might expect a greater response to N inputs in N-limited ecosystems. The coastal sage scrub ecosystem appears to have low natural N availability (Egerton-Warburton and Allen, 2000), perhaps because of frequent fires. Thus, it is likely that these ecosystems are nitrogen

limited. Studies of effects of N fertilization alone on AMF community composition and structure have not been done in communities known to be P limited.

39.3.5 Ericoid Mycorrhizal Fungi and N Deposition

Plants in the Ericaceae typically live in N-limited, organic rich soils (Read, 1991). Some species in this family are declining in areas subject to high N deposition (Bobbink et al., 1998), and Ericaceae can be sensitive to N fertilization (e.g., Prescott et al., 1993). Ericoid mycorrhizal fungi (ErMF) are apparently more capable than other MF at accessing complex organic compounds, supplying otherwise unavailable nutrients to their hosts (Read, 1996; Cairney and Burke, 1998). As a result, one might expect ErMF to be the most sensitive to elevated N inputs. Short-term field fertilization studies of percent colonization have had varying results, with no negative effect in some (Caporn et al., 1995; Johansson, 2000) and ErMF declining in another (Yesmin et al., 1996). A gradient study suggested increasing colonization up to a threshold and then decline (Yesmin et al., 1996). There is no information on community response to elevated N, as ErMF species are difficult to distinguish morphologically, and only recently have the methods of community identification been developed and applied to them (e.g., Allen et al., 2003).

39.3.6 O_3 Effects on Mycorrhizal Community Structure

Although there have been a number of studies on O_3 or other oxidant effects on mycorrhizal fungi, for a variety of reasons they provide limited information on mycorrhizal fungal community responses. First, most studies were limited to examination of mycorrhizal colonization, without regard to the composition of the community. Second, many studies have been pot experiments. These experiments usually lack the full complement of mycorrhizal inoculum, resulting in low colonization percentages compared with natural settings. Third, most studies were carried out using morphotyping with the associated limitations described above. Therefore, interpretation of community response from these studies must be done with caution, and comparisons among studies are quite difficult. As a result, it is impossible to say whether there are any consistent taxonomic responses to increased O_3 exposure. Another difficulty lies with the spatial and temporal scales of exposure, and developmental stage of the plants. Most studies have been done in pots on seedlings, with experiments typically lasting from weeks to months, and only rarely for more than one growing season. The paucity of large-scale, long-term treatments is not surprising, because of the expense and difficulty of such studies. However, given the apparent temporal lags seen in community response to N fertilization (see above), long-term studies are essential to determine mycorrhizal fungal community response to O_3 or other oxidants.

When we add these limitations to the other variables affecting the outcome of experiments noted earlier, it is not surprising that we have limited community response information and find high variability among studies in the effect of O_3 on mycorrhizal fungal infection. Although many studies found decreases in mycorrhizal infection with increasing O_3 exposure, others found no change or increases (Table 39.2). Two factors that seem especially likely to affect mycorrhizal response to the treatments are O_3 concentration and exposure duration. The combination of these, or total dose, appears to be a good predictor of likely response (Väre et al., 1993). Most of the studies that found neutral or positive mycorrhizal responses to O_3 were found in short-term or relatively low-dose experiments. Consistent with this finding, when plants are exposed to a range of concentrations from subambient to ambient to superambient, quadratic response curves can occur (e.g., Stroo et al., 1988), with peak mycorrhizal colonization at or near ambient levels.

Andersen (2003) summarized the hypothesized causes of stimulation of mycorrhizal colonization with relatively short-term low doses. These include mobilization of stored

Table 39.2 Summary of Selected Studies of O_3 Effects on Mycorrhizal Fungal Colonization and Communities

Host/Fumigation System/Experiment Duration	O_3 Treatment: Concentration, Exposure, Timing	SO_2 Treatment	N Treatment	Acidity Treatment (pH)	CO_2	Mycorrhizal Treatment or ID Method	Mycorrhizal Response: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Community Structure: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Reference
Quercus rubra seedlings/growth chambers/52 days	20, 70, 120 ppb		SO_4^{-2}:NO_3^- 2:1 (mass ratio)	3, 4, 5		Total, no ID	Decreased (%) with lower pH (higher N); increased (%) with O_3; interaction with rain pH and soil	N.D.	Reich et al., 1985
Quercus rubra seedlings/open-topped chambers/56 days	Subambient, ambient, 1.5× ambient	0, 16 days @ 90 ppb, 29 days @ 100 ppb				Total, no ID	Decreased (%) with higher SO_2; increased (%) with higher O_3	N.D.	Reich et al., 1985
Betula papyrifera seedlings/closed chambers/84 days	0, 60–80 ppb		SO_4^{-2}:NO_3^- + NH4 10:7 equivalent ratio	3.5, 5.6		± *Pisolithus tinctorius*, ± sterile or natural inoculum, percent infection only	O_3 × pH interaction (%)	N.D.	Keane and Manning, 1988
Pinus strobus seedlings/growth chambers/3.5 months	20, 60, 100, 140 ppb, 3 days/ week		SO_4^{-2}:NO_3^- ratio 2:1	3.0, 3.5, 4.0, 5.6		No ID	Decreased (%, n) with decreasing pH; no O_3 effect	N.D.	Stroo et al., 1988
Pinus strobus seedlings/growth chambers/104 days	20, 60, 100, 140 ppb, 5 days/ week					No ID	Negative or quadratic response (%) to increasing O_3	N.D.	Stroo et al., 1988

Table 39.2 Summary of Selected Studies of O_3 Effects on Mycorrhizal Fungal Colonization and Communities (Continued)

Host/Fumigation System/Experiment Duration	O_3 Treatment: Concentration, Exposure, Timing	SO_2 Treatment	N Treatment	Acidity Treatment (pH)	CO_2	Mycorrhizal Treatment or ID Method	Mycorrhizal Response: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Community Structure: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Reference
Pinus taeda/open-topped chambers/165 days	20, 40, 50, 70, 90 ppb		SO_4^{-2}:NO_3^- 7:3 equivalent ratio	4.0, 5.3		Morphotyping (4 types)	No O_3 effect; increased (n) at lower pH	No O_3 effect; shift in morphotypes (n/cm, n, %) with changing pH; O_3*pH*soil mg interaction for one morphotype	Simmons and Kelly, 1989
Picea abies saplings/closed chambers/14 months	25, 50 ppb			3.0, 5.6, not factorial		Morphotyping	Increased nonmycorrhizal tips with + O_3, + acid mist	No significant treatment effect on morphotypes	Blaschke and Weiss, 1990
Pinus taeda seedlings/cstr[b] chambers/ 42–84 days	0, 50, 100, 150 ppb, 5 h/day, 5 days/week					Morphotyping	Decreased (%) with increasing O_3	Brown morphotype: decrease (n/cm), but no change (%) Coralloid: nonsignificant decrease (n/cm, %)	Meier et al., 1990
Pinus taeda/open-topped chambers/84 days	Ambient, ambient + 80, ambient + 160 ppb		Not reported	3.3, 4.5, 5.2		Inoculated with *Pisolithus tinctorius*	Decreased (%) with high O_3	N.D.	Adams and O'Neill, 1991

Pinus taeda/open-topped chambers/3 years	Subambient, 1×, 2× ambient		SO_4^{-2}:NO_3^- 7:3 equivalent ratio	3.8, 5.2		Morphotypes	Lower (%) in O_3 (p = 0.1)	Lower (%) for coralloid; rhizomorphic type in O_3 (p = 0.1)	Edwards and Kelly, 1992
Pinus rigida/growth chambers/91 days	0, 50, 100, 200 ppb					Inoculated with *Pisolithus tinctorius*	Decreased (%) with increasing O_3; change in ultrastructure	N.D.	Mcquattie and Schier, 1992
Pinus sylvestris and *Picea abies*/open-air fumigation/4 years	16–27 (100)[a], 17–32 (140) ppb	3–6 (60–112)[a], 9–14 (90–226), 13–22 (149–253) ppb				Sporocarp biomass and ID, morphotypes	No treatment effects	No treatment effects	Shaw et al., 1992
Pinus taeda seedlings/open-topped chambers/207 days	0.29×, 1×, 1.71×, 2.38× ambient		SO_4^{-2}:NO_3^- 7:3 equivalent ratio	3.3, 4.3, 5.3		4 morphotypes and *Cenococcum geophilum*	Increase (n/cm) with increasing O_3 for O_3-sensitive pine family	Increase (n/cm) with increasing O_3 for brown, tan morphotypes	Qiu et al., 1993
Picea abies/open-air fumigation/1–2 growing seasons	Ambient, 1.6× ambient					No identification	Increased (n) with higher O_3 (year 1), then decreased (n) (year 2, Ca stressed only)	N.D.	Rantanen et al., 1994
Pinus sylvestris/open-air fumigation/1–2 growing seasons	Ambient, 1.6× ambient					No identification	Increased (n) with higher O_3 (year 2)	N.D.	Ranatanen et al., 1994
Pinus sylvestris saplings/growth chambers/77 days	20, 55 ppb		0, 40 µg m^{-3}		Ambient, 700 ppm	Natural soil inoculum, % infection only	Decreased (%) with higher NH_3, O_3; $NH_3 \times O_3$ interaction	N.D.	Perez-Soba et al., 1995

Table 39.2 Summary of Selected Studies of O_3 Effects on Mycorrhizal Fungal Colonization and Communities (Continued)

Host/Fumigation System/Experiment Duration	O_3 Treatment: Concentration, Exposure, Timing	SO_2 Treatment	N Treatment	Acidity Treatment (pH)	CO_2	Mycorrhizal Treatment or ID Method	Mycorrhizal Response: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Community Structure: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Reference
Pinus halapensis/closed chambers/365 days	0, 50 ppb, factorial with SO_2	0, 40 ppb				Fungi isolated from root tips	Decreased (%) with $O_3 + SO_2$	Reduced coralloid tips (possibly *Suillus*); increased ectendomycorrhizae with elevated $SO_2 + O_3$	Diaz et al., 1996
Picea rubens saplings/open-topped chambers/4 years	0.5×, 1×, 1.5×, 2× ambient		~1.5, 3, 20 kg ha^{-1} $year^{-1}$	3.1, 4.1, 5.1		Morphotypes	No treatment effects	1 year: black type lowest in high pH (low N); O_3 × pH(N) × horizon interaction for 2 types 4 years: one type higher with increasing pH (lower N)	Roth and Fahey, 1998
Pinus sylvestris/open-topped chambers/2.5 years	0×, 1×, ~2× ambient				1×, ~1.7× ambient	4 morphotypes	1.5 years: increase (n) for elevated O_3; CO_2 eliminated O_3 effect 2.5 years: no effect	1.5 years: increase (n) in tuber-like; decrease in dichotomous type for elevated O_3; CO_2 eliminated O_3 effect 2.5 years: no O_3 effect	Kasurinen et al., 1999

Pinus halapensis and *Betula pendula*/growth chambers/68 days	Ambient, 200 ppb		Ambient, 700 ppm	Inoculated *Betula* with *Paxillus involutus*, *Pinus* uninoculated	Reduced fungal growth and higher colonization of *Pinus* with O_3	N.D.	Kytöviita et al., 1999
Pinus sylvestris/open-air fumigation/ 3 years	Ambient, 1.2×–1.7× ambient	32 kg ha^{-1}, 105 kg ha^{-1}		Morphotypes identified but not statistically analyzed	No O_3 effect; elevated N decreased mycorrhizal infection (n)	N.D.	Kainulainen et al., 2000
Pinus halapensis seedlings/growth chambers/94 days	Ambient, 200 ppb		Ambient, 700 ppm	Inoculated with *Paxillus involutus*	No difference (%); decreased soil volume explored with elevated O_3	N.D.	Kytöviita et al., 2001
Elymus glaucus/growth chambers/102 days	~15, ~120 ppm[h]			Inoculated with *Glomus intraradices*	Decreased arbuscules (%)	N.D.	Yoshida et al., 2001
Populus tremuloides and *Betula papyrifera*/open-air fumigation/7+ years	1×, 1.5× ambient		Ambient, 560 ppm	Sporocarp biomass and ID	Decreased sporocarp biomass with elevated O_3, O_3*CO_2 interaction	Decrease in *Leccinum* spp. sporocarp biomass with elevated O_3; increase with elevated CO_2; O_3*CO_2 interaction	Lilleskov, unpublished

Note: Exposures were either to O_3 alone or in combination with a variety of other pollution and soil treatments.

[a] Numbers in parentheses are annual hourly maxima.

[b] Continuously stirred tank reactor.

reserves in roots and increased membrane leakiness, both leading to short-term increases in C availability. Whatever the cause, if indeed low doses of O_3 provide increased C availability to mycorrhizal fungi, this would be likely to have much different effects on mycorrhizal fungal communities than decreased C availability expected as a result of damage to plant shoots. It is therefore critical to elucidate the temporal and dose response curves of O_3 effects on C availability to roots and mycorrhizae over the long term.

Compared with studies on total mycorrhizal colonization, there has been little work on the mycorrhizal fungal community response to O_3. Shifts in morphotype abundance have been seen (Meier et al., 1990; Edwards and Kelly, 1992; Diaz et al., 1996; Roth and Fahey, 1998; Kasurinen et al., 1999; Table 39.2). In several cases, decreases in dichotomous or coralloid morphotypes have been observed (Meier et al., 1990; Edwards and Kelly, 1992; Diaz et al., 1996; Kasurinen et al., 1999). It is unclear whether these always represent species shifts rather than morphological changes within species in response to inputs, although Diaz et al. (1996) found that *Suillus*-like mycorrhizas were being replaced by ectendomycorrhizae under high oxidant loads.

In contrast to N fertilization work, where the vast majority of early information on community response came from sporocarp response to N additions in the field, there are only two studies that have examined sporocarp communities of mycorrhizal fungi under O_3 fumigation. This is due to the much higher cost and effort involved in fumigation compared with fertilization. In the first, Shaw et al. (1992) examined *Pinus sylvestris* and *Picea abies* response to O_3 and SO_2 fumigation with relatively low ambient and additional doses. They found no effects of O_3 and only marginal effects of SO_2 fumigation. The second is an ongoing study of the effects of CO_2 and O_3, so it will be addressed in the following section.

39.3.7 O_3 Interactions with Other Pollutants

Pollutants rarely occur in isolation; therefore, understanding their interactions is critical to our ability to predict net pollutant effects in the field. Effects of some pollutants, e.g., oxidants, might be additive or synergistic. Diaz et al. (1996) found that O_3 or SO_2 alone had only nonsignificant negative effects on mycorrhizal infection or community structure, but in combination they had a larger significant negative effect.

Some pollutants may have antagonistic effects. Interactions between O_3 and acid deposition or soil characteristics (soil type, Ca or Mg concentration, soil horizon) have been observed in some studies for mycorrhizal colonization (Reich et al., 1985; Keane and Manning, 1988; Rantanen et al., 1994) or community structure (Simmons and Kelly, 1989; Roth and Fahey, 1998), but not in others (Qiu et al., 1993; Stroo et al., 1988), suggesting that under some conditions ozone effects on mycorrhizal communities are conditional on soil characteristics and plant nutrition. Similarly, Perez-Soba et al. (1995) found an interaction between the effect of NH_3 and O_3 on percent infection. Although individually they both suppressed mycorrhizal infection, in combination negative effects were eliminated. The cause of these interactions remains to be elucidated and might differ among host genotypes and species, depending on C allocation responses to nutrient addition, their nutrient requirements, and mechanisms of O_3 tolerance or avoidance.

The positive effect of CO_2 on carbon gain could be counteracted by O_3 damage to the foliage and consequent decreases in carbon gain. If CO_2 and O_3 effects on mycorrhizae are mediated solely by carbon gain, then one might expect CO_2 and O_3 combined to result in less change in mycorrhizal community composition than each pollutant individually.

One study examined the effect of exposure to elevated CO_2 and O_3 for 2 years on EMF communities of Scots pine (*Pinus sylvestris*) grown in open-topped chambers in Finland (Kasurinen et al., 1999). Using morphotyping, they found that O_3 exposure led

to an increase in the percentage of roots colonized by a tuber-like morphotype and a decrease in a dichotomous morphotype in the first year; in the next year, exposure to CO_2 led to a decrease in the percentage of roots colonized by a dichotomous, thin-mantled morphotype and an increase in a coralloid morphotype.

We are currently examining the effects of both CO_2 and O_3 on mycorrhizal fungal community structure at the AspenFACE site in Rhinelander, WI (Lilleskov, unpublished). This study, initiated in 1997, is designed to address the ecosystem consequences of CO_2 and O_3 enrichment on the growth of forest trees in a field setting, without the use of chambers (Dickson et al., 2000). Quaking aspen (*Populus tremuloides*) is planted in the 30-m-diameter rings, either alone or in combination with paper birch (*Betula papyrifera*) or sugar maple (*Acer saccharum*). Our preliminary results indicate a significant effect of both CO_2 and O_3 on sporocarp production by mycorrhizal fungi, with significantly lower sporocarp production under elevated O_3, a stimulatory effect of CO_2 on sporocarp production in some cases, but a much stronger CO_2 stimulation seen in combination with O_3. Furthermore, there has been a shift in the species composition of the dominant taxa fruiting, with a positive effect of CO_2 and a negative effect of O_3 seen for some taxa (e.g., *Leccinum* spp.) but not for others (e.g., *Hebeloma* spp.). The sporocarp effects have been quite strong, with virtually no sporocarp production by *Leccinum* in the O_3 treatments, whereas it is the dominant sporocarp biomass producer in the other treatments. We are presently in the process of analyzing the fungal communities on the root tips, to determine whether these changes in production reflect a shift in dominance on root tips or only allocation to sporocarp production.

39.4 MECHANISMS AND CONSEQUENCES OF MF COMMUNITY CHANGE

The consequences of MF community change for plant health and ecosystem function depend largely on whether, as mycorrhizal fungal communities change in response to alterations of resources and conditions, the consequent changes in community function buffer, are neutral with respect to, or exacerbate, the effect of these environmental changes. The answer to this question is fundamental to our understanding both of the mode of control of MF community structure and of the functional role of MF communities in ecosystems. In the following section we will review alternative models of community functional response and the evidence in support of these alternatives.

The core question we need to ask is: Are mycorrhizal communities in any sense optimized in terms of their supply of resources to their plant hosts? A related question relevant to the current discussion is: As resources and conditions change as a result of exposure to pollution or other stresses, do changes in fungal communities lead to new communities that are functionally optimized from the plant perspective? Or alternatively, do changes lead to less mutualistic communities? In order to answer these questions, we must first answer the following question: What do we mean by functional optimization?

39.4.1 What Do We Mean by Optimality?

If we think of optimality in terms of maximizing host fitness, for some herbaceous plants there is the potential to have direct measures of fitness. However, when working with long-lived woody perennials (including many hosts of AMF and almost all hosts of EMF), we must make certain assumptions, because host fitness of a long-lived perennial is very difficult to measure directly in the context of most experiments. Commonly used proxies for fitness are plant nutrition and growth, as both can be influenced by mycorrhizal fungi and are likely to be major determinants of fitness. Caveats for this definition are that high

growth rates may not always be optimal from the perspective of fitness, and defining the optimal nutrient status may be difficult.

39.4.2 How Would Mycorrhizal Fungal Optimal Function Change as N and C Availability Change?

In initially N-limited ecosystems, two aspects of mycorrhizal function are likely to have optima (from the host plant perspective) that shift in response to altered C and N availability: amounts and relative proportions of specific nutrients supplied to host plants, and the C cost of supplying those nutrients. As N availability increases, N limitation will become alleviated, initially leading to greater aboveground plant growth and decreased C flux belowground (Cannell and Dewar, 1994). Given sufficient inputs, other nutrients will become limiting, and these new nutrient limitations will also influence patterns of C flux belowground. Similarly, as C availability declines in response to oxidants, or increases in response to elevated CO_2, C available for nutrient uptake will theoretically be decreased or increased, respectively. Under these conditions, both changes in host requirements for specific nutrients and the availability of host C to the EMF could alter the suites of fungal traits that are functionally optimal.

39.4.2.1 Shifting Optima for Nutrient Supply

Under strongly N-limited conditions, a high affinity for inorganic N and enzymatic capabilities that permit access to complex organic N would both be beneficial, but one might expect that these traits would be less beneficial under high N conditions. Increased N supply can lower host plant demand for N, and high uptake rates of NH_4^+ impose a high C cost on EMF because of the need to incorporate excess NH_4^+ into amino acids, some of which are transferred back to hosts (Wallander, 1995; Wallander et al., 1999). These C costs in combination with nutrient effects on C allocation may contribute to reduced production of external mycelium in fertilized forests (Nilsson and Wallander, 2003). Consequently, traits that lead to reduced uptake of NH_4^+ but favor the uptake of other mineral nutrients should be beneficial to both host plant and EMF under conditions of excess N availability and low root C availability. Similarly, maintenance of extracellular proteolytic activity could also impose unnecessary costs under high N conditions. Limited evidence from pure culture experiments suggests that some dominant fungi under high N conditions grow more poorly with protein as a sole N source than mineral N, in contrast to many fungi from low N sites that grow equally well on protein and mineral N (Taylor et al., 2000; Lilleskov et al., 2002b; Table 39.1). These results require testing on a greater range of isolates and in symbiosis. Intraspecific variability in pure culture growth on protein has also been found (Table 39.1), but it is not known if this variation is related to N availability at the site of origin.

Under elevated N deposition, increased plant uptake, soil acidification, and leaching losses could all lead to reduced base cation and phosphorus (P) availability. If these nutrients become limiting to plant production, the optimality model would suggest that hosts would favor fungal symbionts with traits that maximize the gain of cations or P (e.g., Dighton et al., 1993). These traits could include high-affinity phosphate transporters that allow for uptake of P at low concentrations (Kothe et al., 2002) and increased production of acid phophatases (e.g., Antibus et al., 1997) and organic acids (e.g., Griffiths et al., 1994). The latter would be involved in mobilization of both P and cations.

39.4.2.2 Shifting pH and Mycorrhizal Function

Soil acidification under elevated N (or S) deposition could also lead to shifting optima for mycorrhizal function. For example, Lilleskov et al. (2002a) hypothesized that the decline

of a subset of EMF species over an N deposition gradient might have been driven by acidification rather than N availability per se. Because species function outside optimal pH ranges, nutrient uptake and other functions will be disrupted (Ek et al., 1994), reducing MF ability to be effective mutualists. These pH effects could be mediated by optima for membrane function or extracellular enzyme activity; shifts in forms and decreased availability of nutrients, especially cations and phosphorus; and increases in acid cations, especially Al^{3+}, that could affect root or fungal function. EMF species differ in their sensitivity to Al^{3+} in culture (Thompson and Medve, 1984). Dighton and Skeffington (1987) speculated that the decline in abundance of coralloid mycorrhizal morphotypes in response to high H_2SO_4 could be attributed to higher H^+ or Al^{3+} concentrations. *C. geophilum* appeared to be sensitive to pH in pot studies (Stroo and Alexander, 1985; Meier et al., 1989). Although it exhibited growth tolerance of a wide pH range in pure culture (Hung and Trappe, 1983), it was relatively sensitive to increased Al^{3+} concentrations (Thompson and Medve, 1984), suggesting that Al sensitivity rather than pH per se might be responsible for observed shifts. Ahonen et al. (2003) found differences between Al^{3+} tolerance and growth effects of two ectomycorrhizal fungal species. For a more in-depth treatment of acidification effects, see Dighton and Jansen (1991).

39.4.2.3 Availability of Host C to MF

Oxidative stress reduces C available for allocation to roots (Andersen, 2003). Similarly, allocation theory suggests that plants will respond to alleviation of N limitation by shifting the allocation of C away from the capture of belowground resources to the capture of aboveground resources (Ingestad and Agren, 1991). Source–sink models (Cannell and Dewar, 1994) predict that total belowground C flux will be reduced in response to the alleviation of nutrient limitations to growth because the C sink strength of shoots increases relative to the sink strength of roots. For example, the total quantity of C allocated belowground by stands of *Pinus radiata* and *Eucalyptus saligna* was reduced under conditions of high N availability (Ryan et al., 1996; Giardina and Ryan, 2002). In combination with a reduction in belowground C flux, Giardina and Ryan (2002) observed a dramatic reduction in EMF colonization rates of fine roots. Similarly, oxidant stress reduces the source pool of C, reducing available C for allocation to roots (Andersen, 2003).

However, sufficiently high inputs of N to N-limited ecosystems can lead to the development of limitations by other nutrients, such as P or cations. Of importance to EMF function, P vs. Mg or K limitation has opposite effects on belowground flux of C (Ericsson, 1995). P limitation, like N limitation, results in increased belowground C flux. This C flux can lead to substantial increases in EMF biomass under P limitation (Wallander and Nylund, 1992). In contrast, studies suggest that Mg, and possibly K, limitation results in reduced C flux belowground (Ericsson, 1995). Consistent with this, EM development is restricted under Mg limitation (Ericsson, 1995). Although limitation by these cations is relatively rare, when it occurs there would be limited ways that MF could alleviate limitation because lower C supply to roots might reduce MF ability to produce organic acids to mobilize cations.

39.4.2.4 Integrating Nutrient and C Availability

Thus, altered N availability affects both patterns of nutrient limitation and other aspects of soil chemistry, and the availability of C to obtain those nutrients. Therefore, one might hypothesize that optimal symbionts would shift under N deposition or oxidative stress. As N availability increases, the optimal EMF partner for the host plant would shift, tracking limiting nutrients, soil chemistry, and C availability:

1. Under low N conditions: Aboveground growth is N limited, belowground C flux is high, and fungi effective at supplying inorganic or organic N to hosts, with low to high C efficiency (because C is readily available), are optimal.
2. Under elevated N conditions where nutrition is relatively balanced: Aboveground growth is not nutrient limited, belowground C flux is reduced, and fungi with high C efficiency for uptake of a broad range of nutrients are optimal.
3. Under elevated N conditions where cations such as Mg and K are limiting and soils might be acidified: Belowground C flux is also low (because Mg limitation reduces soluble carbohydrate pools), so fungi with high C efficiency of supplying the limiting cation, possibly in acidified soils, are optimal.
4. Under elevated N conditions where P limitations are encountered, and soils may be acidified: Belowground C flux is again high, and fungi effective at supplying inorganic or organic P, possibly in acidic soils, are optimal.

In the case of elevated oxidants or CO_2, we can expect that the major changes in optimal fungal function would be in terms of the C efficiency of nutrient supply. Under elevated oxidants, more C-efficient fungi would be optimal. Thus, the optimal fungi under oxidant stress might be expected to be most similar to 2 or 3 above in the N deposition scenario, depending on nutrient limitations. The major difference would be that C allocation belowground would be reduced even under N or P limitation, so one would expect the optimal fungi to be those that acquired the most limiting resources, including N, for the lowest C cost.

Under elevated CO_2, fungi with higher rates of nutrient supply with relatively less regard for C efficiency would be optimal, although there would be clear interactions with soil nutrient availability and host limitations described above. The tendency would be for optimal fungi to be more like those under 1 or 4 above, depending on patterns of limitation.

The above discussion assumes that nutrients and C supply are the major controls on root receptivity to fungi. However, it is conceivable that other mechanisms could affect host receptivity to fungi. For example, oxidative stress to foliage could affect mycorrhizas by triggering a systemic induced defensive response against fungi (e.g., Eckey-Kaltenbach et al., 1994) that could inhibit mycorrhiza formation.

39.4.3 Are Mycorrhizal Fungal Communities Optimized as Resources and Conditions Change?

The above discussion of optimality assumes that mycorrhizal fungal communities are selected to optimize plant fitness, nutrition, and growth, and that mycorrhizal associations are predominantly mutualistic across a wide range of environmental conditions. The optimality model suggests that regulatory mechanisms within the host plant result in selection of the most effective (i.e., in terms of supply of limiting nutrients, C costs, or other attributes) fungal mutualists. For example, trees may reduce the supply of plant C to one MF species if there is no nutritional benefit to the host and allocate more to other MF or nonmycorrhizal roots as appropriate. Higher rates of mortality of root tips colonized by poor mutualists vs. good mutualists would lead to increased proportional colonization by more beneficial mutualists (Hoeksema and Kummel, 2003).

However, plant control over which mutualists colonize roots has not been clearly established, and evolutionary models of mutualism suggest that the stability of mutualistic associations depends on the ability of partners to detect parasitism and retaliate (Axelrod and Dion, 1988; Bull and Rice, 1991). The temporal or spatial scales at which plants can recognize and respond to parasitism will affect the fungal fitness consequences of parasitic vs. mutualistic interactions (Hoeksema and Kummel, 2003). Negative host

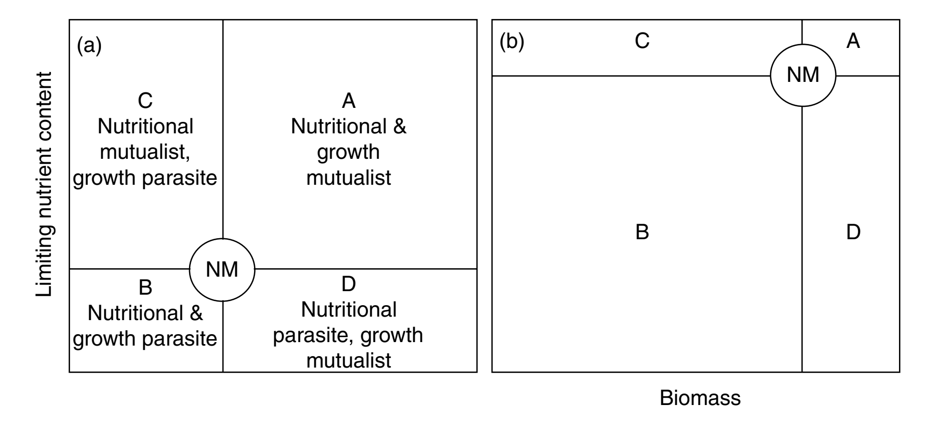


Figure 39.5 Mycorrhizal nutritional and growth mutualism and parasitism under low nutrient availability (a) and high nutrient availability (b). NM = nonmycorrhizal plant. Note that there is less room for mutualism as the fertilized plants approach maximal growth and optimal nutrient concentrations.

fitness consequences of colonization by MF could occur if plant mechanisms controlling choice of fungal partner are weak compared with other factors, such as highly effective root-colonizing ability of relatively parasitic fungi (Eissenstat et al., 1993; Johnson et al., 1997; Bever et al., 2001). In the present context we define parasitic MF as those fungi that provide low or no returns of a limiting resource in exchange for host C supplied, so that plant fitness or its proxies are lower than in the nonmycorrhizal state. Empirical evidence suggests that MF community composition could change in response to fertilization in ways that have suboptimal (but still positive), neutral, or even negative consequences for plant growth (Johnson et al., 1997). Although MF parasitism could theoretically occur under any conditions, it is thought that it should be more likely under fertile conditions. This is because lower belowground C availability and higher nutrient availability could permit nonmycorrhizal plants to achieve uptake of limiting nutrients at a lower cost than mycorrhizal plants (Figure 39.5). Fungal community optimization can only occur if the optimal fungal partner exists. Under extremely nutrient-rich, acidic, or C-limited conditions, the best fungal partner present in the community might not be able to provide resources as efficiently as nonmycorrhizal roots. In this case, the optimal state for the plant would be nonmycorrhizal, whereas the optimal state for the fungus is always in symbiosis. Fungi that are better able to gain access to host C under these conditions (more aggressive strains, *sensu* Johnson, 1993) would by definition be parasitic (Johnson, 1993).

Empirical investigations in AMF plant communities have suggested that fertilization might shift EMF communities toward the parasitic end of the mutualism–parasitism spectrum (Johnson, 1993). Johnson (1993) found that arbuscular mycorrhizal (AM) inoculum from completely fertilized plots, when compared with inoculum from unfertilized plots, had less positive effects on host growth and number of inflorescences. Similarly, Corkidi et al. (2002) compared the relative benefit of inoculum from unfertilized and N-fertilized soils from two sites in the western U.S., when used to inoculate two grass species in either low or high N soils. They found that in both cases, most host parameters measured were improved more by the inoculum from low N soils than those from high N soils, especially when grown in high N soils, and inoculum from high N soils had no significant benefit compared with uninoculated controls (Figure 39.6). Some caution must be used in interpreting this as solely a mycorrhizal effect, because inoculum of pathogenic micro-

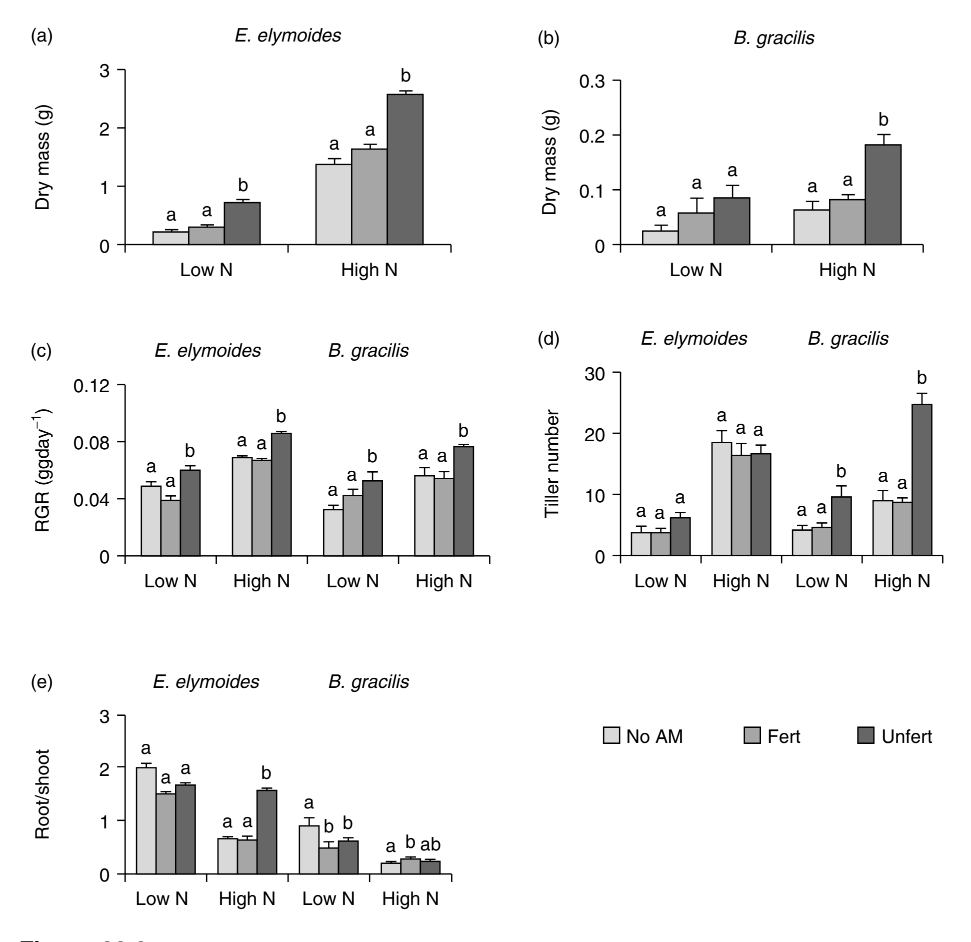


Figure 39.6 Growth response of *Elymus elymoides* and *Bouteloua gracilis* inoculated with fertilized (Fert), unfertilized (Unfert), and nonmycorrhizal (No AM) soil from Shortgrass Steppe, Colorado. Plants were grown in high N and low N conditions for 12 weeks. (a, b) Dry mass. (c) Relative growth rate (RGR). (d) Tiller number. (e) Root/shoot. Bars represent the standard error of the mean of 10 replicates. Within each nutrient treatment, different letters indicate significant differences between soil inoculum treatments at *p* 0.05. Letters above bars of different N treatments cannot be compared. (From Corkidi et al., *Plant and Soil*, 240, 299–310, 2002. With permission.)

organisms could also have differed among soil types. However, the authors report seeing little evidence of pathogens.

It is unclear how general these results are, as no study of this sort has been repeated in EMF or other mycorrhizal fungal communities. Lilleskov et al. (2002a) hypothesized that the increase in abundance of *Paxillus involutus* with increasing N inputs might be the result of a functional shift to fungi adapted to conditions of low pH and P limitation. This species appears to be specialized for high N/low P environments. It is more efficient at inorganic P uptake than N uptake (Ekblad et al., 1995; Högberg et al., 1999), has higher acid phosphatase activity (Pacheo et al., 1991), and supplies relatively more P than N to seedlings than other species, including *Piloderma croceum*, a species sensitive to high N inputs (Wallander et al., 1997; Högberg et al., 1999). Similarly, *Laccaria bicolor* — another species that appears to be relatively tolerant of high N conditions — grows poorly on organic N (e.g., Lilleskov et al., 2002b), provides relatively low amounts of N and high amounts of

P to hosts, and is tolerant of high aluminum availability (Ahonen et al., 2003). These traits could be beneficial under high N conditions and are consistent with an optimization model of community change. However, the generality of these traits, among both different strains and species from high N sites, and the relative benefits of EMF communities from high vs. low N sites under N-enriched conditions remain to be tested. I am aware of no tests of the functional consequences of community change driven by oxidants. This must in part be a consequence of the paucity of data available characterizing community response to oxidants.

In summary, our understanding of the mechanisms and consequences of mycorrhizal fungal community change in response to air pollution is at present quite crude. Future experiments should explore the match between MF communities and the soils from which they are derived, specifically addressing the alternate hypotheses of MF community optimization vs. parasitism under changing resource conditions.

39.5 RECOVERY AND RESTORATION

As pollution abatement measures are implemented, ecosystem perturbation will be reduced, and the potential for community recovery exists. In Europe, economic and technological trends and specific pollution regulation are leading to reduced inputs of N over broad regions (Erisman et al., 2003). What is the potential for recovery of EMF diversity? This question is especially difficult to answer, given that we do not know baseline diversity, the extent of diversity losses, or the mechanisms controlling diversity and composition. If, for example, plant nutrition, soil N availability, and soil pH all affect mycorrhizal communities, then considerable lags are possible in community response, and species-specific patterns of recovery are to be expected. Strengbom et al. (2001) examined plots fertilized with nitrogen 9 and 47 years previously and found patterns indicative of lags in community recovery. Although sampling was limited, there were significant residual effects of N fertilization on sporocarp diversity at both sites, with reduced sporocarp production by *Cortinarius* in fertilized plots at both sites.

One approach to deal with eutrophication effects is active restoration, at least at a small scale. Efforts in the Netherlands involving removal of humus layers from stands with dramatically reduced sporocarp production have resulted in partial recovery of EMF communities that has persisted for 5 years (Smit et al., 2003).

In the case of oxidants, assuming that oxidant effects led to significant community change, one might expect a much more rapid response in the fungal community if, as hypothesized, the primary pathway of pollutant effect is via C allocation. The major factor limiting rates of recovery would likely be the lags involved with host recovery of photosynthetic capacity and species turnover on roots. Consistent with this hypothesis, dramatic reductions in SO_2 and NO_x emissions (88 and 79%, respectively) in the Czech Republic from the late 1980s to the late 1990s have been associated with a parallel dramatic increase in EMF sporocarp production and diversity in the Giant Mountains (Fellner and Landa, 2003). The potential nutrient, acidification, and oxidant effects of these pollutants or their reaction products make it difficult to ascribe the changes strictly to removal of oxidants; however, it is indicative of the potential for relatively rapid recovery from some pollution stresses.

39.6 KNOWLEDGE GAPS

The above should make it clear that air pollution has the potential to alter mycorrhizal fungal communities, with potential biodiversity and functional consequences. However, it

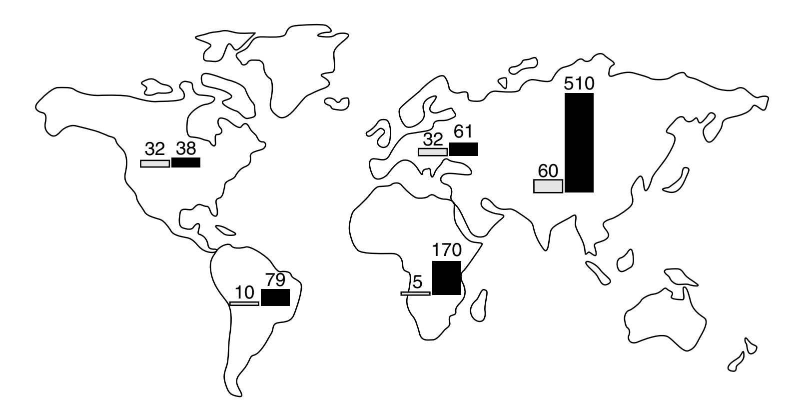


Figure 39.7 Comparison of contemporary and possible future reactive nitrogen creation rates in various regions of the world (1995 vs. max population) (Tg N $year^{-1}$). (From Galloway and Cowling, *Ambio*, 31, 64–71, 2002. With permission.)

should also be clear that in order to assess the full magnitude and significance of community change, we must fill a number of critical gaps in our understanding of the patterns, mechanisms, and consequences of community change. Besides the obvious gaps in our knowledge pointed out above, I wish to highlight several other knowledge gaps that are worth considering as we develop future research programs.

39.6.1 Generality of Mycorrhizal Fungal Community Responses to Pollutants

As should be clear from the earlier discussion of empirical results, there are data from multiple studies suggesting responses of MF communities to N deposition. However, most of this work has been carried out in relatively few ecosystem types: for ectomycorrhizal fungi, most of the work has been done in conifer forests; for AM fungi, most of the work has been done in coastal sage scrub ecosystems (community characterization) or grasslands (community function). Virtually no information is available on the effects of ozone or fertilization on ericoid, arbutoid, and orchidoid mycorrhizal communities. Relatively little work has been done with deciduous trees, including both temperate and tropical ectomycorrhizal and AM tree species. Given the projections for dramatic increases in N deposition in the developing world, especially Asia (Galloway and Cowling, 2002; Figure 39.7), the lack of tropical data is of particular concern. It is possible that the ectomycorrhizal dipterocarps of tropical Asia could be especially sensitive to N deposition.

39.6.2 What Is the Baseline Community?

In order to accurately characterize biodiversity consequences of air pollution, we must have a baseline against which to measure these changes. Unfortunately, for globally mixed pollutants such as CO_2 there is no possibility of establishing a baseline unless archived or otherwise preserved samples can be accessed (e.g., Egerton-Warburton et al., 2001). As noted earlier, current global CO_2 concentrations are ~30% above preindustrial levels. As an added complication, the idea of a baseline for CO_2 may be unrealistic, as even before industrialization the baseline for CO_2 was not stable, but rather varied repeatedly between 180 and 280 ppm during the Pleistocene (Houghton et al., 2001).

In contrast, preanthropogenic levels of atmospheric N deposition and oxidants were low and believed to be relatively stable (Holland et al., 1999). It is still possible to find

regions of the globe at or near preindustrial levels (e.g., Lilleskov et al., 2001, 2002a). However, many fertilization experiments have been carried out in regions already experiencing elevated nitrogen deposition or oxidant concentrations. In these cases, the control communities will not be equal to the baseline, unpolluted communities. Similarly, many studies of oxidant effects use ambient levels as the baseline. However, these studies are often carried out in areas where the baseline O_3 levels are substantially elevated above preindustrial levels. It is essential that studies be carried out in regions with preindustrial levels of oxidants and N deposition in order to establish reliable baselines.

As a result, the amount of good community baseline data is relatively limited, making determination of large-scale pollutant effects more difficult. The best baseline data are from sporocarp records in Europe, made possible by the long tradition of fungal taxonomy there. Monitoring of long-term trends of sporocarp production in Europe was one of the first clues that air pollution might be affecting mycorrhizal fungal communities. Most other regions of the world do not have similar records. If we do not act soon in those areas (especially North America, Asia, Africa, and South America), it may be difficult to find baseline communities for some forest types.

39.6.3 Biodiversity and Biogeography

This lack of extensive baseline data, combined with the challenges involved in species identification for fungi, has limited our knowledge of biogeographic patterns of mycorrhizal fungi. While site-level (alpha) diversity of fungi can be calculated from individual studies, understanding the scales at which both fungal species and air pollutants vary is required to determine the likely regional and global biodiversity consequences of air pollution. Would elevated air pollution likely eliminate genotypes within populations, entire populations, or entire species? Pollutants differ in the spatial scales at which they are elevated. Nitrogen and ozone pollutants have a shorter atmospheric residence time, so they are characterized by local to regional hot spots, with remote areas having relatively low concentrations. As noted above, CO_2 has a longer atmospheric residence time and tends to be relatively well mixed globally, so even remote regions are exposed to elevated CO_2.

In contrast to the patterns of air pollution, we have relatively little information on the biogeographic patterns of species and populations of mycorrhizal fungi (Halling, 2001). In particular, we have very little idea of how much endemism exists in mycorrhizal fungi. Studies suggest high rates of endemism in Australia (Castellano and Bougher, 1994) and New Zealand (McKenzie et al., 2000), and for boletes in Costa Rica and Colombia (Halling, 1996). What about North America, Europe, Africa, and Southeast Asia? Many morphological species appear to be shared across north-temperate regions, but biogeographic differentiation within these species groups is routinely discovered (e.g., Martin et al., 2002). Even within species there is the potential for regional differentiation among isolated populations that needs to be taken into consideration in estimates of loss of biodiversity.

In order to fill these gaps in baseline data, it is critical that large-scale sampling and mapping programs be undertaken to determine mycorrhizal fungal community composition and structure in a broad range of ecosystem types, as is occurring at present in parts of Europe and North America (Arnolds, 2001).

39.6.4 Critical Loads

Critical loads are “a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge” (Nilsson and Grenfelt, 1988). Critical loads for N effects on mycorrhizal fungi have never been estimated. Bouwman et al. (2002) have

calculated critical loads of nitrogen as a nutrient, although these are not based on mycorrhizal fungal responses. They indicate that critical loads for maintenance of biodiversity in response to N eutrophication were exceeded in significant areas of Eastern Europe (47%), Western Europe (38%), the U.S. (24%), and Southeast Asia (23%), based on a medium estimate of critical loads. By 2015, the areas exceeding these critical loads are projected to decline for Europe, stay stable for the U.S., and increase to 30% for Southeast Asia.

Can we say what a likely critical load for nitrogen deposition would be for mycorrhizal communities? Wallander and Kottke (1998) suggested that a critical load of 20 to 30 kg ha^{-1} $year^{-1}$ could be too high for sensitive EMF communities. If we can link changes in mycorrhizal communities to specific processes already addressed by critical load models, such as changes in nitrification, then we might be able to use them to refine current models to predict critical loads for mycorrhizal fungal diversity. Increase in net nitrification is believed to be an important indicator of significant N eutrophication (Aber et al., 1998). Most studies of mycorrhizal fungi have not linked community response to specific N cycling parameters such as nitrification. Taylor et al. (2000) and Lilleskov et al. (2001, 2002a) (Figure 39.3 and Figure 39.4) found negative correlations between diversity of EMF and various metrics of soil inorganic N pools. More studies must establish links between N cycling parameters and mycorrhizal community diversity, composition, and structure before we can determine appropriate critical loads for EMF diversity. Metrics of this sort are likely to be more useful predictors than nitrogen inputs because the latter do not take into account the other factors that could influence N cycling, such as soils, site history, climate, productivity, and vegetation.

39.7 CONCLUSIONS

There is good evidence that nitrogen deposition is one of the major factors contributing to decline in diversity of EMF sporocarps over broad regions of Europe. Although there is less available evidence, experiments and gradient studies suggest that sites with long-term N inputs are also losing diversity belowground. Much less good MF community information is available for oxidant effects. Although lab experiments suggest that oxidants can have negative effects on mycorrhizal fungi, there is a need for field experiments that address long-term oxidant effects at realistic concentrations. More extensive biogeographic data are necessary to assess the full biodiversity impacts of mycorrhizal fungal community change. Similarly, functional consequences of community change are largely unknown. Limited research with AMF communities indicates the potential for fertilization-mediated transitions to communities of less beneficial mycorrhizal fungi, but the generality of this phenomenon across mycorrhizal classes and plant communities must be determined. The development of DNA-based tools has provided us with an unprecedented opportunity to characterize community composition and structure. This fundamental community information will allow us to make meaningful inroads into the complex questions related to mycorrhizal community function. Clearly, there are many urgent and exciting challenges ahead for the current generation of mycorrhizologists.

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40

Micromycete Associations in the Rhizosphere of Steppe and Agrophytocenose Plants

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40.1 INTRODUCTION

Basically all biogeocenoses that exist on the surface of Earth, including nondisturbed steppes, are influenced by man. In the Ukraine all that remain are a few unique nondisturbed steppes with total territory less than 1% of the whole steppe zone (Tkachenko et al., 1998). Natural steppe ecosystems are composed of unique and specific plant species; they have an abundant animal and microbial world. Detailed analysis of natural reserves as ecological and biological models of biocenoses became very important with the many anthropogenic changes imposed on the environment.

It is well known that fungi play an important role as indicators of an ecosystem's stability (Berestezki et al., 1980; Zhdanova and Vasilevskaya, 1982; Moore, 1988; Hawksworth, 1998). Soil is the natural ecotope of many fungi where they often start and end their life cycle. Formation of micromycete complexes under specific ecological conditions is influenced by environmental factors, each of which exerts specific influences on the abundance of the individual species. Plant cover is one major factor influencing micromycete populations in soil (Moore, 1988). The changes in rhizosphere mycobiota depend on the root exudates and decomposing plant residues.

Investigations of the natural diversity of micromycete reserves in soils and the specific soil fungi adapted to life in various ecological environments are very important when anthropogenic influences are significant.

40.2 MATERIALS AND METHODS

The major objective of our research was to study the species composition and fungal complexes in the rhizospheres of vegetative communities in three regions of the Ukrainian Steppe Natural Reserve (USNR): Mykhailivska Tsilyna, Khomutovskyi Step, and Kamyani Mohyly. These communities have similar soil and climatic conditions and are used for permanent winter wheat monoculture, as well as sugar beet and corn production. Multifactoral field experiments have been carried out in the Mironovka Institute of Wheat Breeding since 1926. Our experiments were conducted during 1994–1997. Soil samples were taken from 0 to 15 and 15 to 30 cm depth (Zvyagincev et al., 1984).

40.2.1 Isolation of Fungi from Soil

Soil micromycete studies were conducted as previously described (Waksman and Fred, 1922). In short, 10 g of soil was placed in 90 ml of sterile water and shaken for 5 min. A series of dilutions 1:10, 1:100, and 1:1000 was made and plated on Czapek's solution agar containing 50 mg/l of streptomycin. Plates were incubated at 25 to 27°C, and colonies were isolated and transferred to agar plates and grown for another 3 to 21 days for further examination, characterization, and identification. Taxonomic literature was used for identification of the fungal isolates to species (Booth, 1971; Ellis, 1971; Bilai, 1977; Domsch et al., 1980; Hawksworth et al., 1995).

Comparison of the micromycete species composition for different experiments was conducted according to the Sorensen–Czekanowski formula $S = 2c/(a + b)$, where c represents the number of common species, a the number of species in the soil of one sample, and b the number of species in the soil of another sample (SPSS, 1990).

To estimate the level of species diversity we used the index of Shannon, $H = -\Sigma(n_i/N)\log(n_i/N)$, where n_i is the frequency of occurrence of each species and N is an estimate of significance (100%).

The Simpson's index of domination was determined according to the formula $C = \Sigma(n_i/N)^2$, where n_i is the frequency of occurrence of each species and N is an estimate of significance (100%) (Odum, 1986).

The number of fungal colonies per gram of dry soil was determined according to the formula $A = a.b.c.\ g^{-1}$, where a represents the average number of colonies per Petri dish, b the dilution series from which colony isolation and counting was done, c the mass of the wet soil, and g the mass of the dry soil.

40.3 RESULTS AND DISCUSSION

Our results showed that among the soil ecotypes we studied, differences existed in both fungal species composition and number of species and strains. For example, we isolated and identified 65 species (317 strains) in Mykhailivska Tsilyna, 56 species (260 strains) in Khomutovskyi Step, and 51 species (209 strains) in Kamyani Mohyly, which belong to 25 genera and 3 sections (Zygomycota, Ascomycota, mitosporic fungi).

The majority of fungal species in soil of Mykhailivska Tsilyna was found in the rhizosphere soils of *Euphorbia stepposa* Zoz. (20 species), *Phlomis tuberosa* L., and *Poa aiqustifolia* L. (17 species in each); whereas *Filipendula vulgaris* L., *Stipa pennata* L., and *Thalictrum minus* L. showed a lower number (16 species in each).

The rhizospheres of multigrass typical-feather-grass association and multigrass narrow-leaf–thin-stem–feather-grass association in Khomutovskyi Step showed a wide diver-

sity of fungal species (27 for each). The rhizospheres of other plants had fewer species: 20 in *Linosyris villosa* (L.) DC and *Euphorbia stepposa* Zoz., 19 in *Limonium platyphyllum* Lincz., and 18 in *Salvia nutans* L.

The Kamyani Mohyly plant community had a different number of soil fungi, with *Centaurea pseudoleucolepis* Kip. and *Lathyrus tuberosus* L. harboring the highest number of species (19 and 18, respectively). Other plant species showed lower numbers of fungal species.

Mykhailivska Tsilyna characteristically had a wide diversity of species. The rhizospheres of steppe plants in this community showed a significant number of genera (6) and species (11) of the Zygomycota section, of which species of the Mucoraceae family were the most abundant. Members of this family composed 52.8% of all fungal species in Mykhailivska Tsilyna, 10.5% in Khomutovskyi Step, and 9.2% in Kamyani Mohyly. Genera *Mucor* and *Rhizopus* (three species each) and *Absidia* (two species) were the most numerous of the Mucoraceae family. In all cenoses we found *M. hiemalis* Wehmer, *M. mucedo* Linnaeus: Fr., *M. racemosus* Fres., *R. stolonifer* (Ehrenb.: Lk.), *R. orysae* Went. et Prins., and *A. spinosa* Lendn. Wide spreading of these fungi can be explained by their ability to metabolize different carbohydrates. The Mortierellaceae family is represented in the rhizosphere of steppe plants of Mykhailivska Tsilyna by three species: *Mortierella alpina* Peyr., *M. polycephata* Coem, and *M. stylospora* D. Stewart. Two species of this family (*M. alpina* and *M. polycephata)* were found in Khomutovskyi Step, and only one in Kamyani Mohyly (*M. alpina*).

Section Ascomycota was represented by six species: *Melanospora zobelii* (Corda) Fuck., *Chaetomium globosum* Kunze: Fr., *Eupenicillium meridianum* Stolk et Scott, *Emericella nidulans* (Eidam) Vuill., *Eurotium herbariorum* (Wigg.: Fr.) Lk, and *Eurotium rubrum* Konig et al.

Genus *Penicillium* was the most widely spread (32.3%). Species of this genus dominated in all experiments because they are generally the most typical soil microorganisms. According to Koval (1984), a small number of representatives of the *Asymmetrica* section are the characteristic feature of the reserve soils, whereas they usually dominate in cultivated soils. *P. chrysogenum* Thom, *P. glabrum* (Wehmer) Westl., *P. raciborskii* Zaleski, *P. janczewskii* Zaleski, and *P. varians* G. Sm. were isolated rather often from all territories of USPR. On the other hand, such species as *P. lanosocoeruleum* Thom, *P. multicolor* Novobr., and *P. purpurogenum* Stoll were typical only for the soil of Mykhailivska Tsilyna. It is also important to note the availability of large numbers of colorless and light-colored species. Domination of the members of the *Penicillium* genus can be explained by their high spore-forming capability and high adaptability to unfavorable environments. They can easily tolerate extremely high and low temperatures; their spores remain viable in highly toxic soils and can germinate at low humidity.

Seven species (13.2%) belonged to genus *Fusarium*: *F. gibbosum* (App. et Wr.) Bilai, *F. javanicum* Koord., *F. moniliforme* Sheld., *F. oxysporum* Schl.: Fr., *F. sambucinum* Fuck., *F. solani* (Mart.) Sacc., and *F. sporotrichiella* v. *poae* (Pk.) Wr. Ement Bilai. Species of this genus are notably variable and ecologically flexible, mostly due to aggressive fermentation complex, which allows them to metabolize different substrates (Bilai, 1984).

Members of the genus *Aspergillus* fungi were quite abundant (7.6%) in all experiments. The most abundant in steppe soil were *A. fumigatus* Fres., *A. niger* van Tiegh., *A. terreus* Thom, and *A. versicolor* (Vuill.) Tirabochi. According to the recent data summarized in Marfenina (2002), distribution of this genus in soils and on technogenic materials is always correlated with human activity.

It is also important to mention species that are rare among fungal biota: *Melanospora zobelii*, *Eupenicillium meridianum*, *Mortierella stylospora*, *Acremonium rutilum* W. Gams,

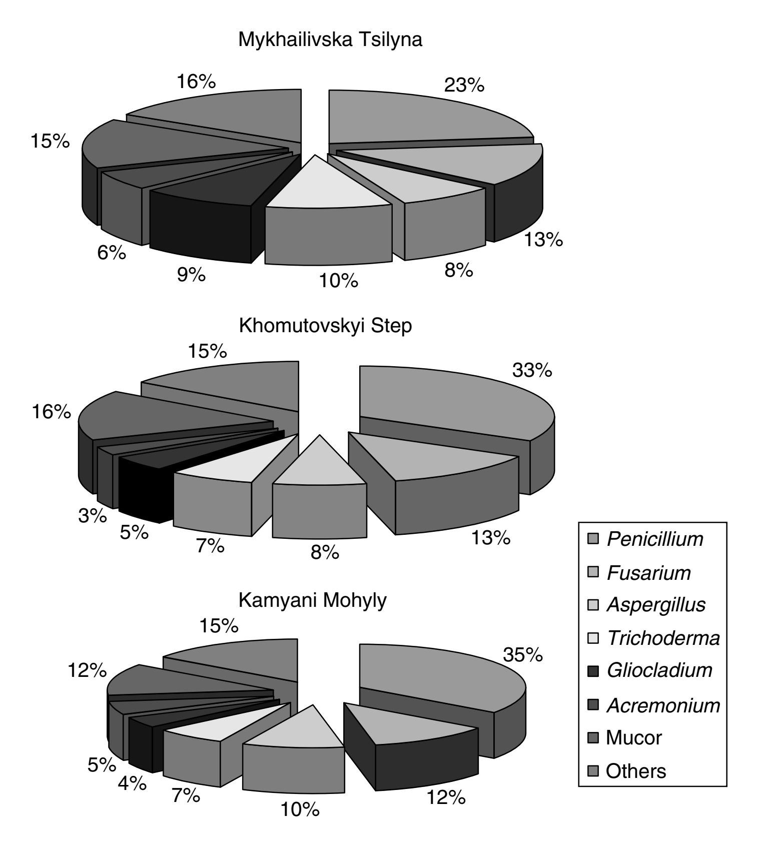


Figure 40.1 **(See color insert following p. 460.)** Correlation of dominating groups of soil micromycetes in Ukrainian Nature Steppe Reserve.

Mucor mucedo, *Doratomyces stemonitis* (Pers.: Fr.) G. Sm., *Gliocladium catenulatum* Gilm. et Abbott, *Penicillium multicolor* Novobr., *P. variabile* Sopp, and *Trichoderma state of Hypocrea aureoviridis* Pers.: Fr. Melanin-containing species amounted to 19%.

Dominant species were *Mortierella alpina*, *Acremonium strictum*, *Alternaria alternata* (Fr.: Fr.) von Keissl., *Aspergillus niger*, *Fusarium oxysporum*, *Gliocladium roseum* Bainier, and *Trichoderma viride* Pers.: Fr., distribution frequency for which exceeded 50%.

The Khomutovskyi Step showed a reduced number of species (56), compared with Mykhailivska Tsilyna, where 65 species were found. Species from the section Zygomycota were limited; however, species of more typical genera of other sections were not significantly different. This pertains to the species of genera *Acremonium*, *Fusarium*, and *Cladosporium*, and the majority of the genera *Penicillium* and *Trichoderma* species (Figure 40.1).

The list of *Aspergillus* species had only one additional member — *A. ustus* (Bain.) Thom et Church. The following species were not found in the Khomutovskyi Step: *Botrytis cinerea* Pers.: Fr., *Gliocladium catenulatum*, *Humicola grisea* Traaen, and *Scopulariopsis brevicaulis* (Sacc.) Bainie; however, they were isolated from Mykhailivska Tsilyna soil. The share of melanin-containing species was 17.8%. Among the species that were rare in Khomutovskyi Step, it is important to mention *P. dierckxii* Biourge and *P. vulpinum* Seif. et Samson in addition to the above-mentioned fungi. *Fusarium oxysporum*, *Gliocladium roseum*, *Trichoderma viride*, and *Penicillium raciborskii* were dominant fungi (see Figure 40.2).

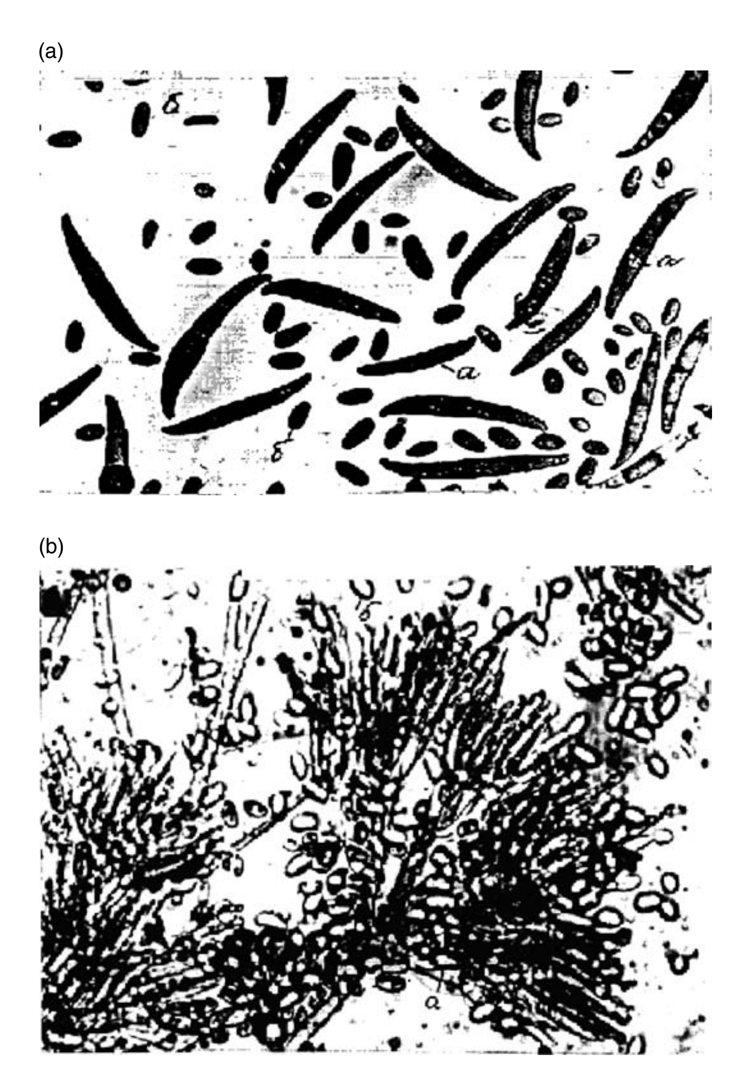


Figure 40.2 Species dominating in Ukrainian Nature Steppe Reserve: *Fusarium oxysporum* Schlecht.: Fr. (A) and *Gliocladium roseum* Bainier (B).

40.4 KAMYANI MOHYLY

During our study of Kamyani Mohyly we found smaller numbers of species (51) than in other regions. The species composition of the Zygomycota section was reduced significantly. The number of genus *Penicillium* species here was at par with the other regions (20), and 15 of them were common for all three experiments. Five species represented genus *Aspergillus*; all of them except *A. wentii* Wehm. were found in other locations. Five species represented genus *Fusarium*. Species of the genus *Trichoderma* (typical soil inhabitants) were isolated from all ecotopes during the entire vegetation period. *T. viride* was the most abundant. Frequency of occurrence of this fungus was 81.8% in Mykhailivska Tsilyna, 71.4% in Khomutovskyi Step, and 61.5% in Kamyani Mohyly. Fungal population of the steppe ecosystems included not only *T. viride*, but also *T. koningii* Oudem and *T. state of Hypocrea aureoviridis.*

Table 40.1 Frequency of Occurrence of Soil Fungi in UNSR Regions

Regions of the Reserve	Dominant (>50%)	Frequently Found (30–50%)	Regularly Found (10–30%)	Few Occurrences (1–10%)	Occasional (<1%)
Mykhailivska Tsilyna	6	10	33	16	—
Khomutovskyi Step	4	23	29	—	—
Kamyani Mohyly	3	30	28	—	—

To summarize, the majority of micromycete species isolated from rhizospheres of the steppe plants are typical saprotrophs that take an active part in biological processes occurring in soil.

Data about diversity of micromycete species in the rhizosphere of steppe plants in various UNSR locations became the basis for data generalization and further analysis. Fungal species were distributed among three groups based on frequency of occurrence (Table 40.1):

1. Dominant species (frequency of occurrence higher than 50%) — These species included: in Mykhailivska Tsilyna, *Mortierella alpina*, *Acremonium strictum*, *Alternaria alternata*, *Fusarium oxysporum*, *Gliocladium roseum*, and *Trichoderma viride*; in Khomutovskyi Step, *Fusarium oxysporum*, *Gliocladium roseum*, *Penicillium chrysogenum*, *Penicillium raciborskii*, and *Trichoderma viride*; and in Kamyani Mohyly, *Fusarium oxysporum*, *Gliocladium roseum*, and *Trichoderma viride*.
2. Frequently found species (frequency of occurrence 30 to 50%) — They included *M. mucedo*, *Rhizopus oryzae*, *Acremonium rutilum*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium solani*, *Fusarium moniliforme*, *Gliocladium varians*, *Penicillium chrysogenum*, and *Trichoderma koningi* in Mykhailivska Tsilyna. In Khomutovskyi Step and Kamyani Mohyly, species from this group were more abundant (23 and 30, respectively).
3. Regularly found species (frequency of occurrence 10 to 30%, see Table 40.1). There were more species that belong to this group in Mykhailivska Tsilyna (33). Also, there were many such species in Khomutovskyi Step and Kamyani Mohyly (29 and 28, respectively). The role that these species play in the ecosystem should not be underestimated because they enrich special diversity of any ecological niche.

Values for the Sorensen–Czekanowski coefficient of similarity of species composition of microscopic fungal complexes, Shannon's general diversity, and Simpson's domination determined in our studies are shown in Table 40.2 and Figure 40.3. Comparison of both dominant and total species pointed out the high similarity of species compositions of soil fungi among all three locations.

40.5 MICROMYCETES IN CULTIVATED SOILS

Experience and scientific experiments show that higher plants and soil microorganisms interact through root exudates and products of mineralization of harvest residues. Along

Table 40.2 Sorensen–Czekanowski Coefficients of Similarity for Microscopic Fungi Complexes in Rhizosphere of Steppe Plants in Different UNSR Locations[a]

Location	Mykhailivska Tsilyna	Khomutovskyi Step	Kamyani Mohyly
Kykhailivska Tsilyna	+		
Khomutovskyi Step	0.78/0.57	+	
Kamyani Mohyly	0.74/0.41	0.81/0.50	+

[a] The numerator is the coefficient of similarity based on frequency of occurrence of all species. The denominator is the coefficient of similarity based on frequency of occurrence of dominant species.

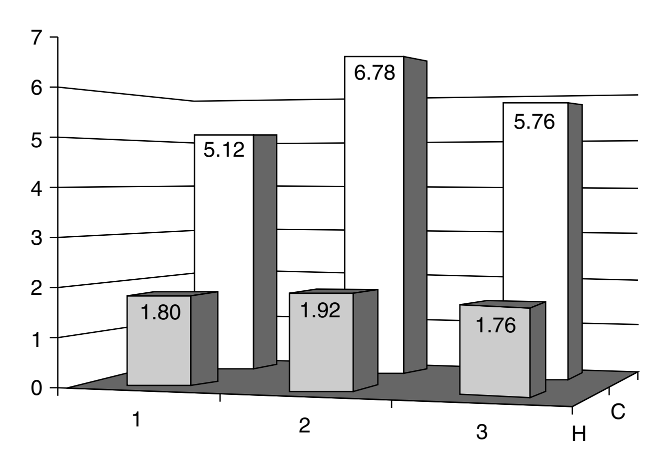


Figure 40.3 Shannon's coefficient (H) and Simpson's index of domination (C) in Mykhailivska Tsilyna (1), Khomutovskyi Step (2), and amyani Mohyly (3).

with a positive role that the microbiota plays in the natural phytocenoses, it is also important to note the phytoxicity of soils in agrophytocenoses. Application of fertilizers, tillage of harvest residues under the soil surface, preference for wheat, sunflower, and sugar beet in crop rotation schemes, especially when they are used as monocultures — all this significantly changes the structure and functions of microbe communities (Golovko, 1997). Many Ukrainian research institutes (Zabolotny Institute of Microbiology and Virology, Grishko National Botanical Garden of Sciences of Ukraine) have been studying cultivated soils under different agricultural crops (wheat, corn, sugar beet, alfalfa, etc.) for many years. According to our previously published data (Golovko, 1984), root exudates from crop plants stimulate the dynamics of fungi (see Table 40.3).

The Shannon coefficient is the most frequently used for analysis of soil microbiota ecological data (Mirchink, 1988). It can be seen from Figure 40.3 that all studied soils had insignificant Shannon coefficients as follows: Mykhailivska Tsilyna, 1.81; Khomutovskyi Step, 1.92; and myani hyly, 1.76. This indicates the low level of species diversity. It is well known that the values of this coefficient are highest in tropical forests, moderate in agrocenoses, and lowest in undisturbed steppes.

Contrary to low species diversity, the Simpson's index of domination was high in all experiments. According to Odum (1986), the stronger the domination of one or a few species, the higher the Simpson's index. The share of dominant species was very significant

Table 40.3 Seasonal Changes of Microscopic Fungi Number in Chernozem (thousands per gram of soil)

	I Term			II Term			III Term		
Date	**31/5**	**7/7**	**22/10**	**4/6**	**1/7**	**19/9**	**30/4**	**31/7**	**17/10**
				Monoculture					
Winter wheat	54.0	8.5	33.0	89.9	25.8	91.0	57.0	30.8	33.3
Corn	8.3	63.0	48.4	62.3	14.0	56.1	26.4	17.5	34.8
Long-term fallow	2.4	15.0	41.0	5.3	4.2	4.9	60.9	14.8	35.8
Undisturbed	28.7	74.0	33.0	38.3	50.1	45.8	53.6	46.3	63.1
				Rotation					
Winter wheat	24.0	12.6	35.9	61.1	10.4	54.1	15.6	5.6	26.1
Corn	20.2	13.0	47.8	34.5	15.8	24.3	24.0	11.4	50.1
Sugar beet	28.7	14.6	55.1	18.7	6.7	19.4	18.9	22.0	19.9
Perennial grasses	33.8	10.7	22.4	69.3	62.3	55.1	24.2	51.7	36.0

compared to the total number of soil fungi (Figure 40.3). Our data thus support the conclusion that ecosystems under periodical anthropogenic influence develop higher species diversity compared to untouched ones with significant domination and low competition (Odum, 1986).

Micromycetes were four to six times more abundant at all stages of development of winter wheat and corn as monocultures relative to crop rotation. Soil under undisturbed steppe and perennial grasses showed relatively high fungi content at all vegetation stages. Species composition in the rhizosphere of plants in crop rotation significantly differed from that of permanent culture (Golovko, 1984).

We also compared the lists of fungi species identified in one of our locations — Mykhailivska Tsilyna — with literature data on micromycetes found under barley and oat (Kirilenko, 1984). Results were expressed as the Sorensen–Czekanowski index of similarity (0.32), which indicates a significant difference between undisturbed and cultivated soils. Soil cultivation significantly influences the species composition of fungi. It is expressed in notably lower frequency of occurrence of some *Mucorales* species, in particular genera *Mortierella* and *Aspergillus.* At the same time, the structural complex of micromycetes of soils under cereals is characterized by high numbers of *Fusarium* and *Gliocladium* species; the significant number of melanin-containing hyphomycetes also appears (Table 40.4).

To summarize, the distribution of micromycete species in the rhizosphere of steppe plants in various locations of UNSR under different soil and climatic conditions and under agricultural crops was thoroughly studied. The most significant species diversity was found in Mykhailivska Tsilyna (65), compared with Khomutovskyi Step and Kamyani Mohyly (56 and 51 species, respectively). Species composition of the most typical genera did not significantly differ among all locations of the reserve. It applies to species of genera *Acremonium*, *Fusarium*, *Cladosporium*, *Penicillium*, and *Trichoderma*. The number of Zygomycota fungi was reduced at southern locations, Khomutovskyi Step and Kamyani Mohyly. Composition of Ascomycota species was more or less the same at all locations of the UNSR. The number of melanin-containing species was not significantly different — 19% in Kykhailivska Tsilyna, 17.8% in Khomutovskyi Step, and 15.7% in Kamyani

Table 40.4 Soil Micromycetes under Winter Wheat

Monoculture	Rotation
Zygomycota, Mucorales	**Zygomycota, Mucorales**
Mortierella alpina Peyr.	*Mortierella alpina* Peyr.
Absidia corymbifera (Cohn) Sac. et Trott.	*M. polycephata* Coem.
Mitosporic fungi	*M. stylospora* D. Stewart
Aspergillus sclerotiorum Huber	*Absidia corymbifera* (Cohn) Sac. et Trott.
A. ustus (Bain.)Thom et Church	**Ascomycota, Sordariales**
Aspergillus sp.	*Chaetomium globosum* Kunze: Fr.
Fusarium sp.	**Mitosporic fungi**
Gliocladium roseum Bainier	*Aspergillus ochraceus*
Penicillium lanosum Westl	*Fusarium graminearum* Schwabe
P. varians G. Sm.	*F. oxysporum* Schl.: Fr
Penicillium sp.	*Gliocladium catenulatum* Gilm. et Abbott
Trichoderma hamatum (Bon.) Bain	*G. roseum* Bainier
T. viride Pers.: Fr.	*Penicillium cinereo-artrum* Chalab.
	P. cremeo-griseum Chalab.
	P. dierckxii Biourge
	P. funiculosum Thom
	P. janczewskii Zaleski
	P. rugulosum Thom
	P. variabile Sopp
	P. viridicatum Westl.
	Torula herbarum
	Trichoderma koningii Oud.

Mohyly — which indicates the insignificant anthropogenic alterations in these regions. Investigated steppe biogeocenoses are characterized by similar species composition of soil fungi despite different soil climatic conditions. Agricultural use of soils significantly changes the structure and functions of microbial communities.

Therefore, the use of the methods described above for ecological evaluation allows comparisons, both quantitative and qualitative, of the compositions of soil mycobiota, which in turn provide excellent insight into the character and level of anthropogenic transformation of soils.

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41

Fungal Communities of Agroecosystems

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41.1 INTRODUCTION

The term *agroecosystem* encompasses all components in an agricultural landscape, including crops and pastures, livestock, other flora and fauna, soil, water, and the atmosphere (U.S. EPA, 2003). Associations among components are dynamic, and within the context of the term, there is an awareness that management of crops or livestock will have impacts on other ecosystem components and their functions. In general, agricultural practices represent large-scale anthropogenic disturbances that alter one or more ecosystem components and their association with other components. For example, disturbances to soils, via cultivation and fertilizer inputs, will alter the physical structure, nutrient availability, and resource heterogeneity of soil environment. This in turn will affect the fungal community residing in that particular environment, and possibly their roles in ecosystem functioning. The degree to which fungal community structure or function is altered will be dependent on the severity and frequency of disturbance, but if the heterogeneity of the environment is decreased, imposed environmental homogeneity will presumably reduce fungal species richness and diversity (Zak, 1992).

Despite recent efforts to qualify and quantify the impacts on agricultural practices on biological indicators of soil quality (for a review, see Doran and Zeiss, 2000), we know surprisingly very little regarding the impacts to soil fungal communities. One exception may be the arbuscular mycorrhizal (AM) fungi, where their responses to agricultural stresses and their contributions to soil conservation and crop productivity have been relatively well studied and reviewed (Bethlenfalvay, 1992; Johnson and Pfleger, 1992; Miller and Jastrow, 1992, 2000). In general, however, soil fungal *communities* are understudied in agricultural sciences. In the previous edition to this book, only a handful of studies were

presented in the brief section describing soil fungal responses to agroecosystem disturbances. There, Zak (1992) presented cases where tillage and monocropping reduced fungal species richness and altered the species composition of the fungal community, but the effects on fungal activities such as nutrient cycling and organic matter accumulation were largely unknown at the time. In a more recent review, Miller and Lodge (1997) summarized effects of tillage and crop rotations on soil fungi, with an emphasis on pathogenic and mycorrhizal populations. They also outlined several significant functional roles of soil fungi in agroecosystems, namely, stable aggregate formation and nutrient cycling, and thereby illuminated the importance of fungi to the stability of agroecosystems.

The challenges ahead of soil microbiologist and mycologists are several. Many microbiologists who work in agricultural settings (including myself) were trained or taught from a bacterially dominated perspective, and we must acknowledge the importance of fungi and include them in our investigations of soil microbial diversity in agroecosystems. We must emphasize the importance of fungi to the public and to our predecessors. We must identify alternative and sustainable cropping systems that enhance and sustain fungal community populations in soil. We must examine in more detail the linkage between community function and community species composition. Specifically, we must address disturbance impacts on fungal community composition and determine the degree of compositional changes that will result in significant functional shifts. In this chapter, I will review functions of fungi that are important to soil conservation and crop productivity in agroecosystems, major types of agricultural stresses and their impact on saprophytic and AM fungi, and mechanisms by which fungal community structure and diversity may be enhanced and maintained through conservational/sustainable practices. I will focus on more recent studies, including those with molecular applications, and I will emphasize linkages between fungal species composition and specific functions that impact agroecosystem processes.

41.2 FUNGAL CONTRIBUTIONS TO AGROECOSYSTEMS

41.2.1 Soil Aggregation, SOM Accrual, and C Sequestration

One important role of fungi in soil ecosystems is that of soil conservation through aggregate formation, a mechanism by which C can become physically protected from microbial attack and thus sequestered in soil. Numerous studies have found that fungal hyphal length and other indicators of fungal biomass/necromass are positively associated with the percentage of macroaggregates, including water stable aggregates (WSAs) in soil (Beare et al., 1997; Chantigny et al., 1997; Guggenberger et al., 1999b; Schutter and Dick, 2002), and that the role of fungi in soil aggregate formation, particularly when hyphal densities are high, can be more important than that of bacteria (Chantigny et al., 1997). As fungal hyphae extend throughout the soil matrix, their adhesion to soil particle surfaces initiates the formation of microaggregates (Beare et al., 1997). The soil-exploring mycelium has been described as a skeletal (Miller and Jastrow, 1992), cross-linking (Degens et al., 1996), "sticky string bag" (Miller and Jastrow, 2000) that physically enmeshes soil particles and organic debris together. Stabilization of aggregates occurs as particles are cemented together by extracellular polysaccharides or other "glues" derived from fungal hyphae, other microorganisms, and root exudates. Finally, fungal hyphae physically enmeshed microaggregates to form small macroaggregates, and small macroaggregates to form larger macroaggregates, which are stabilized by more microbial cementing agents (Miller and Jastrow, 1992).

Two important stabilization agents derived from fungi are glucosamine, a component of the cell wall material chitin, and glomalin, which is specific to AM fungi. Glucosamine

is relatively stable in soil, has a longer turnover time than that of living hyphae (Guggenberger et al., 1999b), and appears to be an important binding agent that stabilizes aggregate structure (Chantigny et al., 1997; Guggenberger et al., 1999a, 1999b). Accumulation of hyphal cell wall residues, as measured by glucosamine content in soil, was associated with an increase in the weighted mean aggregate diameter (WMD) in a field study under no-tillage management (Guggenberger et al., 1999b). In a laboratory study where soil was amended with starch, substrate-enriched microhabitats acted as hot spots of fungal growth toward and on these sources (Guggenberger et al., 1999a). The resulting filamentous entanglement of primary particles and microaggregates formed macroaggregates, but because microbial biomass declined shortly after soil amendment (4 days), stabilization of macroaggregates was attributed to more recalcitrant compounds, including fungal cell wall residues, which persisted long after hyphal death. A second, recently discovered compound is glomalin. Glomalin is a hydrophobic glycoprotein produced in copious amounts by AM fungal hyphae (Wright and Upadhyaya, 1996, 1999). During the life cycle of the AM fungus, glomalin is sloughed from the hyphae, where in the soil it behaves as a water-insoluble glue to stabilize soil aggregates. Field studies have demonstrated strong, positive correlations between glomalin content in soil and soil aggregate stability (Wright et al., 1999; Wright and Anderson, 2000). The purpose for which glomalin serves the AM fungus is unknown, but glomalin production likely constitutes a large carbon cost to the organism. Curiously, glomalin's purpose may involve habitat modification. In a laboratory study, researchers found that glomalin production by *Glomus intraradices* was linked to growing space conditions (Rillig and Steinberg, 2002). AM fungi were cultured in transformed, hairy roots in dishes containing small or large glass beads to simulate either a nonaggregated or aggregated soil, respectively. Under nonaggregated conditions, fungal hyphal lengths were reduced by 80%, but glomalin concentrations were seven times greater than under aggregated conditions. The authors proposed that glomalin production may be a mechanism by which AM fungi modify their soil environment when conditions for hyphal growth are suboptimal. Alternatively, when hyphal growth is rapid, more fungal C is allocated to hyphae at the expense of C allocation toward glomalin production.

Aggregate formation and stabilization by fungi can have important consequences with regards to C flow and sequestration. For example, particulate organic matter (POM) is often found at the core of microaggregates, where it is protected from rapid decomposition by encrustation with inorganic material. POM originates from root, hyphae, and fecal matter debris and becomes incorporated into macroaggregates through physical or biological means (Miller and Lodge, 1997). Furthermore, significant amounts of soil C are stored in empty (nonliving) hyphal filaments. As indeterminate and explorative organisms, often the majority of fungal tissue in soil is nonliving and consists of empty hyphal filaments. Fungal C is stored in the cell walls of these empty filaments, mainly as chitin. Because chitin is more slowly decomposed than biomass of other organisms, fungal cell wall residues represent a relatively stable form of soil organic matter (Stahl et al., 1999). In addition, filamentous fungi provide a major pathway of C flow into soil via translocation of surface litter or plant photosynthate C to soil. In a laboratory study using ^{13}C-labeled wheat straw, Frey et al. (2003) measured significant transfer of litter-derived C from the soil surface into underlying soil by decomposer fungi. Litter-derived C was ultimately deposited into newly formed macroaggregates (>250 μm), and the amount of litter-derived C found in macroaggregates was positively correlated with litter-associated fungal biomass. Thus, fungal-mediated litter-to-soil C transfer is an important mechanism by which litter C enters soil and becomes stabilized as organic matter within macroaggregates.

Because AM fungi account for a significant amount of plant photosynthate (up to 20%), extraradical hyphae represent another substantial pathway for C flow into soil. The

amount of C allocated to the soil varies, but there is evidence that AM fungi can affect the balance between plant productivity and soil quality by influencing photosynthate allocation to hyphae and ultimately soil. For example, a greenhouse study demonstrated that the C balance between pea and *Glomus mosseae* shifts when pea is planted in different soils. Specifically, seed yield of pea was unaffected, but the percentage of WSAs increased by 400% in one soil, whereas in a second soil, seed yields increased by 56% while soil aggregation increased only by 50% (Bethlenfalvay and Barea, 1994). Differences in root colonization, hyphal extension in soil, and differences in soil texture and nutrient contents may be important factors affecting the ability of AM fungi to control carbon allocation from host plants to hyphae. More recently, photosynthate transfer to and turnover time in AM fungal hyphae were determined under semisterile greenhouse conditions (Staddon et al., 2003). Almost all of the C photosynthate imported into *Glomus* hyphae from *Plantago lanceolata* was replaced in 6 days or less, suggesting a relatively rapid turnover of extraradical mycorrhizal hyphae in 5 to 6 days. While most of the photosynthate C will be quickly recycled back to the atmosphere as CO_2, some of the C will be sequestered in the soil as cell wall materials of empty hyphae, aggregate stabilizing agents, or physically protected POM.

On various levels, the abilities of soil fungi to stabilize aggregates and sequester soil C are not functionally redundant. For example, fungi differ in the types and quantities of aggregate-stabilizing "glues" they produce, as demonstrated by the ability of AM fungi, but not saprophytic fungi, to produce glomalin, as well as the amount of glomalin produced by different AM fungal species, which ranges from 17 to 63 $\mu g\ mg^{-1}$ hyphae (Wright et al., 1996). Within AM fungal communities, there is evidence that different species of AM fungi have different abilities to produce extraradical hyphae, which can influence the development of soil aggregates. Specifically, biovolumes of *Gigaspora gigantea* have been shown to be positively correlated with hyphal length and soil macroaggregation in contrast to *Glomus* spp., which were negatively associated with extraradical hyphal lengths and the percentage of soil macroaggregates (Miller and Jastrow, 1992; Schreiner and Bethlenfalvay, 1995). Therefore, agronomic practices that cause a shift in AM fungal community structure have the potential to impact the functional significance of the mycorrhizal community in terms of aggregate formation. An example of such a practice is the conversion from conventional tillage to no-tillage, which may allow for tillage-sensitive species, including *Gigaspora* spp., to recover and persist.

41.2.2 Nutrient Acquisition and Cycling

Soil fungi have important functional roles in nutrient acquisition and cycling that distinguish them from soil bacteria. First, fungi typically make up the largest percentage of biomass of the soil community (Lowell and Klein, 2001), and as a consequence, soil fungi are major contributors to soil nutrient cycling processes, including denitrification and N mineralization-immobilization transformations (Laughlin and Stevens, 2002). Secondly, saprophytic fungi are important decomposers of organic materials in soil, including recalcitrant, nitrogen-poor plant polymers and lignin. Fungi are well adapted to colonizing nutrient-poor and recalcitrant substrates in part due to their wide-ranging enzymatic capabilities and efficient allocation of nutrients into vegetative and reproductive tissues. In addition, the indeterminate lifestyle of filamentous fungi provides a distinctive advantage over bacteria due to the recycling and allocating of nutrients from older hyphal regions to regions of active growth. Fungal mycelial growth, in general, appears to be regulated to maximize hyphal spread through the soil matrix, allowing the organisms to colonize multiple substrates in different soil regions simultaneously (Paustian and Schnürer, 1987b). As resources are depleted from a substrate colonized by older hyphae, cytoplasm is

translocated from the older hyphae to areas of new growth. A consequence of this type of hyphal organization is that filamentous fungi can colonize a recalcitrant substrate and translocate nutrients, including N, to the substrate from spatially separated soil microsites enriched in mineral N. It appears that filamentous fungi can translocate nutrients between spatially separated resources over considerable distances, including the translocation of mineral N from nutrient-enriched soil microsites to N-poor residues at the soil surface (Frey et al., 2000).

Mycorrhizal associations benefit host plants in several ways, such as by increasing resistance of plants to water stress (Ellis et al., 1985) or diseases (Sharma et al., 1992), but perhaps the best-described contribution of AM fungi to host plant growth is due to uptake of soil nutrients, particularly P, via extraradical hyphae (George et al., 1995). The AM mycorrhizal hyphae form a continuum of soil solution from the bulk soil to the rhizosphere and the rhizoplane and into the root itself. Because the length ratio of fungal hyphae to roots in soil can be quite high (100:1 or higher; George et al., 1995), the extraradical hyphae are able to explore and scavenge for nutrients in areas of the bulk soil spatially separated from nonmycorrhizal plant roots. Nutrients are then translocated through the hyphae to arbuscules within cortical cells of the plant root. This intimate association between plant roots and AM fungi can be highly effective, and laboratory studies have shown that more than 70% of the plant P content can be due to P uptake by hyphae (George et al., 1994). However, AM fungi are not functionally redundant, despite that a given plant species can be colonized by a number of AM fungal species. In reality, individual species of AM fungi differ markedly in their abilities to take up and translocate nutrients and, thus, in their abilities to affect individual plant hosts. For example, estimates of length-specific hyphal P uptake per unit time range from 1 to 430 fmol P m^{-1} s^{-1} among AM fungal isolates (Jakobsen et al., 1992). Based on growth chamber studies, *Glomus intraradices* and *Glomus caledonium* have greater P uptake rates than *Glomus invermaium*, whereas *Scutellospora calospora* has lower rates of P uptake than *G. invermaium* (Schweiger and Jakobsen, 2000). In addition, AM fungal isolates have different capacities to transport P across soil distances. Hyphae of *Acaulospora laevis* have been shown to spread faster and further in soil than hyphae of *Glomus* sp., and thus *A. laevis* has the ability to transport P over longer soil-root distances (Jakobsen et al., 1992). At least this particular functional compatibility can be influenced by host plant species, as demonstrated in the case of *G. invermaium*, which can transport significant amounts of P when associated with flax, but not with cucumber or wheat (Ravnskov and Jakobsen, 1995).

Importantly, AM fungal isolates differ in their mycorrhizal efficiencies, or the amount of P translocated to the plant per amount of plant C used by the fungus. *Glomus caledonium*, for instance, translocated more P to and consumed less C from cucumber than *S. calospora* or another *Glomus* sp., and thus has a greater mycorrhizal efficiency than the other two species for this particular host (Pearson and Jakobsen, 1993). *Glomus intraradices* has also been identified as an inferior mutualist, capable of draining hosts of C under certain conditions (Johnson and Pfleger, 1992). Douds and Millner (1999) raised the question of whether AM fungi abundant in high-nutrient, well-fertilized soils are plant growth promoters or more aggressive than other AM fungi at acquiring host carbon. Eight years of fertilization of a low-nutrient soil caused four species, including *G. gigantea*, to decline and *G. intraradices* to increase (Johnson, 1993). When big bluestem grass was inoculated with AM fungal communities from fertilized soil, growth was reduced compared with grass inoculated with nonfertilized AM fungal communities, presumably due to greater C drain (Johnson, 1993). Under microscopic examination, roots inoculated with AM fungal communities from nonfertilized soil had fewer arbuscules, the site of nutrient transfer to the host. Hart and Reader (2002) investigated whether a relationship existed

among AM fungal taxonomy, colonization strategy, and mutualistic benefit. Based on the colonizing behaviors on leek of 21 AM fungal isolates, representing species of Glomaceae, Gigasporaceae, and Acaulosporaceae, on leek, most Glomaceae isolates colonized leek roots before species of Gigasporaceae or Acaulosporaceae. The amount and proportion of fungal biomass in roots and soil differed among the families; *Glomus* isolates had relatively high root colonization but low soil colonization, whereas *Gigaspora* isolates showed the opposite trend. Distinct AM fungal colonization strategies existed that were related to taxonomic differences at the family level. AM fungi regenerating primarily from spores (i.e., *Gigaspora* isolates) were the slowest colonizers, whereas *Glomus* spp. demonstrated the fastest colonization rates due to their ability to colonize new roots from old root fragments containing hyphae. Compared with *Glomus* isolates, species of Gigasporaceae may be more mutualistic because they provide the most nutritional benefit for their hosts due to a heavy investment in primary absorptive hyphae. A high ratio of soil-to-root colonization may provide the host plant with the greatest benefit, due to increased potential for nutrient uptake by extensive, absorptive extraradical hyphae. However, low root colonization may reduce the incidences of arbuscules and, thus, limit nutrient transfer to the root (Hart and Reader, 2002).

Finally, it should be noted that the functional significance of a diverse AM fungal community extends well beyond aggregate formation and stabilization, P transfer, and mycorrhizal efficiency. There are important species differences in terms of the ability of different AM fungi to protect host plants from environmental stresses. For example, in the case of drought stress, wheat inoculated with *Glomus fasciculatum* produced more tillers after severe-level water stress than noninoculated wheat or wheat inoculated with *Glomus deserticola* (Ellis et al., 1985). Similarly, Klironomos et al. (2001) found that AM fungal species responded differently to water stress, whereby drought increased root colonization for some plant–fungal combinations, with decreased root colonization in others, further supporting the hypothesis that distinct functional groups of AM fungi exist.

41.3 AGRICULTURAL STRESSES

41.3.1 Tillage

Tillage practices are conducted to modify the soil so that its conditions are more conducive to plant growth. Tillage serves several purposes, including seedbed preparation and aeration, incorporation of residues or chemicals, and weed control. With conventional tillage, soils typically are inverted by a moldboard plow during the primary tillage operation. This action buries most of the residues from the previous crop, usually leaving <30% of the surface covered by residues. To fungi, tillage practices are physical disturbances that redistribute food resources and fungal spores, disrupt hyphal networks, and destroy ecological niches via loss of soil structure.

Soil inversion can be a detriment to fungal communities as food resources and fungal spores are mixed throughout the plow layer. In nondisturbed soils, where plant residues remain on the surface, saprophytic fungi have a competitive advantage over bacteria because their hyphae can extend from the soil across air gaps to the surface. Surface residues become colonized by saprophytic and facultative pathogenic fungi, and as a result, fungi become the dominant microbial community component (Miller and Lodge, 1997). However, when residues are incorporated, soil bacteria are allowed access to the substrates, and the microbial community shifts toward a greater bacterial component. A second consequence of soil inversion is the redistribution of fungal spores away from the surface. For AM fungi, this can result in reduced colonization rates and phosphorus uptake by

crops as AM fungal spores and hyphae are redistributed away from the rooting zone of young plants (Johnson and Pfleger, 1992).

Filamentous fungi generally are sensitive to physical disturbances such as cultivation practices, which are often observed to decrease lengths of fungal hyphae in soil (McGonigle and Miller, 1996; Klein and Paschke, 2000; Lowell and Klein, 2001). While physical mixing of the soil may reduce hyphal lengths directly via hyphal fragmentation, hyphal lengths may also be shorter as the result of homogenized resources that allow fungi to utilize newly available nutrients in their immediate environment without the need for extensive growth (Lowell and Klein, 2001). Arbuscular mycorrhizal fungi are particularly sensitive to fragmentation of hyphal networks because many species rely on extraradical hyphae as inocula for the colonization of new roots. Johnson and Pfleger (1992) speculated that tillage would impose a strong selection pressure on AM fungi, favoring species that rapidly produce infective propagules. Although molecular-based studies are limited, there is evidence to suggest that genetic diversity of AM fungi in intensively cropped systems is low and that the fungal community tends to be dominated by *Glomus* species (Helgason et al., 1999; Daniell et al., 2001). It appears that many species of *Glomus* are more tillage resistant than other genera because of their enhanced ability to fuse disconnected hyphae and quickly reestablish an interconnected mycelial network following mechanical disturbance (Daniell et al., 2001). In contrast, other genera of AM fungi, including *Gigaspora* and *Scutellospora*, may only be capable of propagation via spore dispersal or infection from an intact (i.e., nondisturbed) mycelium (Klironomos and Hart, 2002).

When examining the combined impacts of tillage on soil microbial community structure, tillage-induced disturbances may be a greater detriment to fungi than bacteria. Stahl et al. (1999) compared fungal biomass between cultivated and virgin or long-term (>80 years) prairies and found significant reductions in active and total fungal biomass in cultivated soils. While total microbial biomass declined with tillage as well, it was found that the community structure shifted to a greater proportion of bacterial biomass, with fungal biomass C levels falling from 32 to 34% of the total microbial biomass C pool in noncultivated soils to 20% in cultivated soils. On a finer scale, recent studies have been conducted to characterize the impacts of tillage on the structure of AM fungal communities. Oehl et al. (2003) collected and identified AM fungal spores from different grasslands and arable soils. They were able to characterize individual species as generalists or specialists based on their occurrence at all sites or within a specific ecosystem, respectively. More than half of the 37 species identified were specialists of the species-rich grasslands, and only one species (*Glomus aggregatum*) was restricted to the high-input continuous maize monocropping fields. Eight other species (*Glomus mosseae*, *G. geosporum*, *G. albidum*, *G. etunicatum*, *G. diaphanum*, *G. constrictum*, a species of the *G. fasciculatum* group, and *S. calospora*) were found in all field sites, and of these species, *G. mosseae*, *G. geosporum*, and *G. etunicatum* dominated the arable soil communities. Overall, spore abundance and species diversity was reduced in the cropped sites compared with grasslands. In addition to changes in AM fungal community structure, communities of the different ecosystems exhibited different ecological strategies. Based on trap culture experiments, AM fungi of grasslands had rapid root colonization but slow sporulation rates, whereas AM fungi of continuous maize soils colonized roots slowly but sporulated rapidly. The authors hypothesized that AM fungi that sporulate rapidly and within a single growing season are able to survive intensive farming practices better than slower-sporulating species (i.e., those that did not sporulated within 8 months of the trap culture experiment). Molecular-based studies have shown a similar trend. When AM fungal DNA was extracted, amplified, and sequenced from roots of various woodland plants and crop species, the genetic diversity of AM fungi *in planta* was found to be much less in the

arable sites than in woodlands (Helgason et al., 1998; Daniell et al., 2001). In roots of pea, maize, and wheat, 92% of all sequences detected were represented by *G. mosseae* or closely related taxa (Helgason et al., 1998). Again, this species sporulates rapidly and colonizes readily from spores and, thus, would be favored in systems that are regularly disturbed by tillage.

41.3.2 Monocropping and Fallowing

Interactions between plants and microorganisms are known to create positive- and negative-feedback loops that influence both plant and microbial community structure (Klironomos, 2002, 2003; Bever, 2003). For example, interactions between a specific plant and soil microorganisms in the plant rooting zone can result in a shift in microbial community structure toward a predominance of pathogens, which would inhibit plant growth (negative feedback), or beneficial arbuscular mycorrhizal fungi, which would enhance plant growth (positive feedback). As a result of plant–microbial interactions and feedback loops, species composition of microbial communities can differ among rhizospheres of different plant species (Grayston et al., 1998; Ibekwe and Kennedy, 1998), and even among cultivars of the same species (Siciliano et al., 1998). This appears to hold true within specific populations of microbial communities, including AM fungi. Traditionally, interactions between AM fungi and plants are thought to be of low specificity because of the ability of individual AM fungi to colonize many plant host species. However, greenhouse and field studies have revealed optimum host–fungus combinations whereby fungal spore production is enhanced under a specific plant (Bever et al., 1996, 2001; Douds and Millner, 1999). In a mixed grass and forbs field, for example, community dominance under *Allium vineale*, field garlic, was due to *Acaulospora colossica*, whereas *S. calospora* dominated under *Plantago lanceolata*, narrow-leaf plantain (Bever et al., 1996).

When forest and grasslands are converted for agriculture use, vegetation diversity is dramatically altered, particularly in monocropped systems, where a single crop species is grown in space and over time. Loss of plant species diversity lowers microbial niche heterogeneity as the diversity of plant substrates, root architecture, and host plants declines. Under the selective pressures exerted by the environment and remaining plant species, fungal communities will become dominated by certain rhizosphere fungi and host-specific fungal symbionts and pathogens. Disturbingly, there is evidence to suggest that monocropped systems may even select for aggressively colonizing AM fungi that are less efficient or even pathogenic (Miller and Lodge, 1997), whereby crops receive little benefit due to poor P translocation efficiencies or excessive C drainage by the symbionts. Johnson et al. (1992), for example, observed that AM fungi dominant under continuous corn (*G. mosseae*, *G. occultum*, *G. aggregatum*, and *G. leptotichum*) contributed to yield declines over time. In continuous corn fields strip-planted to corn or soybeans, numbers of corn-associated spores were negatively correlated with the yield of the following corn crop, but positively correlated with yields of succeeding soybean.

Absence of plant roots altogether via extended fallowing can be a severe detriment to mycorrhizal communities as a whole, as well as cropping with nonhost plants. Nonhost crops include plants of the chenopod and crucifer families, such as spinach, kale, sugar beets, canola, and mustards (Miller and Lodge, 1997). Both situations can lower mycorrhizal populations and reduce colonization potentials in succeeding crops by as much as 50% (Black and Tinker, 1979; Johnson and Pfleger, 1992; Gavito and Miller, 1998a; Miller and Lodge, 1997). In addition, loss of residues to soil ecosystems, by either crop removal or fallowing, will reduce the quantity of substrate resources for saprophytic fungi and, thus, lower soil fungal biomass overall (Stahl et al., 1999).

41.3.3 Fertilizer Additions

Nitrogen (N) and phosphorus (P) inputs are known to impact soil fungal community active biomass and species composition. When considering filamentous fungi in general, it is important to remember that fungi are indeterminate microorganisms; they exist as hyphal networks with variable boundaries, whereby cytoplasm is moved to new areas of hyphal extension as older hyphae are abandoned (Klein and Paschke, 2000). The proportion of cytoplasm-filled hyphae (active hyphae) to total hyphae is affected, among other factors, by the quantity and quality of available nutrient resources. With fertilization disturbances, the increase in available soil nutrients can result in an increased allocation of carbon to cytoplasm synthesis at the expense of hyphal development, based on the concepts presented by Paustian and Schnürer (1987a). For example, studies on a semiarid soil found that even though N inputs to a semiarid soil did not affect total fungal hyphal lengths, the proportion of cytoplasm-filled to total hyphae increased (Klein et al., 1989; Klein and Paschke, 2000). Species composition of fungal communities can also be impacted by fertilization effects. In a study of native and recently cultivated semiarid soils, Lowell and Klein (2001) found markedly different fungal community structures between N-fertilized and nonfertilized plots. Based on molecular analysis of 18S rRNA gene sequences, *Phaeosphaeria nodorum*, fungi from the order Pleosporales, and fungi from the domain Basiodiomycota were common to and dominant in the N-amended sites, despite cultivation history. *Phaeosphaeria nodorum*, a pathogen of wheat, produces stress-resistant spores and is capable of growing on several forms of N, including NO_3^- and NH_4^+ (Halama et al., 1999), which may provide a competitive advantage over other fungal species in disturbed and high N environments. Specific impacts on species composition of AM fungal communities have been reviewed by Johnson and Pfleger (1992), with additional case studies by Douds and Millner (1999). These reviews present evidence that fertilization tends to increase the relative spore abundance of *G. intraradices* in soil, whereas populations of *Gigaspora margarita*, *G. gigantea*, and *S. calospora* decline in response to fertilizer amendments (Thomson et al., 1986; Douds and Schenck, 1990; Johnson, 1993).

41.4 CONSERVATION OF FUNGI THROUGH SUSTAINABLE PRACTICES

41.4.1 Reduction in Soil Disturbance

Conversion from conventional tillage to no-tillage management should remove strong selective pressures, such as soil homogenization and structural degradation, associated with conventional tillage. With a reduction in soil physical disturbance, one should expect to see longer lengths of fungal hyphae in soil and an increase in the proportion of fungal biomass relative to bacterial biomass, especially in no-till soils where levels of organic matter, an important food source for saprophytic fungi, are increasing. This was supported by Beare et al. (1997), who reported that lengths of fungal hyphae in surface soil under long-term no-tillage were 1.3 to 1.5 times longer than fungal hyphal lengths under conventional tillage. Moreover, differences in active fungal hyphae were even more pronounced than those of total hyphae, ranging from 2.3 to 3 times higher in no-tillage plots than in conventionally tilled plots. Increases in fungal cell wall materials and other signature compounds are also indicative of improvements in fungal biomass in response to no-tillage conversion. Levels of glucosamine, for example, were higher in field soils under no-tillage than under conventional tillage at several locations in the Great Plains (Guggenberger et al., 1999b). Generally, twofold higher ratios of fungal:bacterial cell wall materials were detected in no-tilled soils where soil organic matter levels were also

increasing in response to no-tillage. However, glucosamine levels were unchanged in soils with a shorter duration of no-tillage and where gains in organic matter had not yet been achieved. The relative abundance of arbuscular mycorrhizal fungi is also predicted to increase in soil after conversion from conventional tillage to reduced or no-tillage. Several studies have demonstrated a positive response of AM fungi upon conversion to no-tillage, based on greater concentrations of glomalin or the fatty acid biomarker for AM fungi, 16:1ω5*c*, in no-tilled soil than in tilled soil (Wright et al., 1999; Drijber et al., 2000; Wright and Anderson, 2000). Often, inoculum potentials and root colonization are higher in no-tilled or reduced tilled vs. conventionally tilled agroecosystems. This has been shown for maize during early-season growth (McGonigle and Miller, 1993; McGonigle et al., 1999; Mozafar et al., 2000; Galvez et al., 2001a), winter wheat (Galvez et al., 2001b), and overwintering cover crops such as hairy vetch (Galvez et al., 1995). Higher levels of AM fungi in no-tilled soils were sometimes functionally significant, such as the increase in macroaggregate stability due to higher glomalin contents in no-tilled soil (Wright et al., 1999). But higher inoculum potential and percent root colonization do not guarantee higher crop yield in no-till compared with conventionally tilled systems. Soils under no-tillage tend to have cooler temperatures, which can depress crop yields despite enhanced inoculum potential and colonization of crop roots (Galvez et al., 2001a). Also, crop yields in several studies did not benefit from improved mycorrhizal associations because soil P levels were well above that at which a yield response to increased P availability was expected (McGonigle and Miller, 1993; McGonigle et al., 1999; Galvez et al., 2001b).

With conversion from conventional tillage to reduced or no-tillage practices, one may also expect that the reduction in physical disturbance will change the species composition of fungal communities, especially in AM fungal communities for which tillage-resistant and tillage-sensitive species have been identified. In one field study, 13 years of long-term reduced tillage did not impact AM fungal spore diversity overall, but a shift in species composition based on spores was detected; non-*Glomus* AM fungal spores, including spores of *Gigaspora*, *Scutellospora*, and *Entrophospora*, were detected more frequently in no-tilled soil, whereas in conventionally tilled soil, AM fungal spores almost exclusively belonged to *Glomus* spp. (Jansa et al., 2002). Differences in *Glomus* species composition based on tillage regimes have also been detected. For example, Douds et al. (1995) reported that spores from the *G. occultum* group were more numerous in no-tilled soil, but *G. etunicatum* and other *Glomus* spp. type spores predominated in tilled soil.

41.4.2 Cropping Rotations

Just as the cessation of intensive tillage should reduce selective pressures on soil fungi, so should the incorporation of different crops into a rotation. Crop rotations allow for an increase in plant diversity over time, which, for example, should increase the heterogeneity of microbial niches via more diversified substrate resources. In addition, fallow phases can be replaced by vegetative phases, which benefit saprophytic fungi by providing substrate resources that otherwise would not be there during that particular phase. An overwintering cover crop also provides additional substrate resources that can stimulate soil fungi and increase their relative abundances (Bossio et al., 1998; Schutter et al., 2001). Or, in the case of AM fungi, an overwintering cover crop can serve as a host to sustain AM fungal populations prior to establishment of the summer cash crop. In a study focusing on AM fungi, Boswell et al. (1998) found that a winter wheat cover crop increased AM fungal inoculum potential of a field soil for the following growing season. As a consequence, root colonization of maize was enhanced in the succeeding maize crop, ultimately resulting in better growth and higher yields than maize grown in plots without a winter cover crop. Within no-tillage field plots in a Great Plains soil, root colonization by AM

fungi was greater in winter wheat of the wheat–corn–fallow and wheat–corn–prosso millet rotations than in winter wheat of the wheat–fallow rotation. Differences in wheat root colonization between the wheat–corn–fallow and wheat–fallow treatments were significant, for example, during early stages of wheat growth (43 and 19%, respectively). By early dough stage, however, root colonization was significantly reduced in the wheat–corn–prosso millet rotation compared with other rotation treatments (9 vs. 16% for wheat–corn–fallow), suggesting that individual crop species may be an important factor affecting AM fungi in high-intensity cropping systems (Stromberger, unpublished data).

The choice of crops to add in rotation can be important, especially for AM fungi if the crop is nonmycorrhizal. In a field experiment, previous cropping with canola (nonmycorrhizal) reduced shoot P concentration and shoot growth of maize at early stages (Gavito and Miller, 1998b). When preceded by maize in another rotation treatment, however, maize roots were rapidly colonized by AM fungi, which resulted in greater root P uptake, shoot P and greater maize yields than yields achieved when canola was the preceding crop. Similarly, P uptake, percent root colonization by AM fungi, shoot weight, and grain yield were much higher in maize following a mycorrhizal crop (sunflower, soybean, potato, or maize) than in maize following fallow or a nonmycorrhizal crop (canola or sugar beet) (Arihara and Karasawa, 2000). The effects of preceding crops on maize growth were at least partly due to differences in AM fungal spore density caused by various preceding crops, which then affected root colonization by AM fungi (Karasawa et al., 2002).

By increasing the diversity of plant species in a given soil over time, crop rotations have the potential to influence the diversity and species composition of AM fungal communities. In a study comparing low-input vs. conventional systems, the diversity of AM fungal spores was shown to be proportional to vegetation diversity, with greater spore diversity occurring in the low-input system that had at least five different crop species in rotation than in a conventional rotation consisting of only two crops (Douds et al., 1993). *Gigaspora gigantea*, in particular, was more abundant in the diverse, low-input system than in the conventional system. Oehl et al. (2003) also found that the diversity of AM fungal spores was significantly greater in fields cropped to a 7-year rotation than in fields continuously monocropped with maize. *Glomus* spp. predominated in continuous maize fields, whereas species of *Acaulospora* were present in rotation fields in addition to *Glomus*. Slowly sporulating species, including *G. invermaium*, *Acaulospora laevis*, and *Scutellospora pellucida*, were absent in monocropped sites, which had short periods of vegetation and prolonged fallow periods, but were present in fields with crop rotations that included pasture and overwintering cover crops in the rotation. Moreover, the species richness of AM fungi in the organically managed crop rotation system approached that of seminatural grasslands.

The above discussions have focused on the diversity of crops in rotation, as well as whether crops in the rotation include mycorrhizal or nonmycorrhizal host species. This chapter would be remiss, however, if it did not include a discussion of mycorrhizal responsiveness within cultivars of a particular crop species. It has been found that cultivars of many agronomic crops, including wheat, corn, and soybean, demonstrate varying levels of colonization by AM fungi, and the degree to which cultivars are colonized is a heritable trait selectable through plant breeding (Johnson and Plfeger, 1992). One hypothesis for cultivar differences in mycorrhizal responsiveness is that crop breeding programs typically select for high-yielding varieties under fertilized conditions. Because mycorrhizal benefits are generally reduced under high-fertility conditions, such crop breeding programs may inadvertently select for genotypes unresponsive to AM fungi in the field. This phenomenon has been studied in both wheat and maize. In wheat, mycorrhizal responsiveness is fairly strong and consistent in standard height–weight varieties released prior to 1975, whereas

many modern semidwarf varieties released after 1975 lack significant response to mycorrhizal fungi, despite similar levels of root colonization (Hetrick et al., 1995). For some modern varieties, mycorrhizal associations can actually depress growth of wheat, demonstrating that mycorrhizal symbiosis may not guarantee benefits to host plants independent of soil and environmental conditions (Hetrick et al., 1992, 1993, 1995). Based on these studies, the authors proposed that selective wheat breeding practices under conditions of chemical inputs may have resulted in the loss or suppression of genes responsible for mycorrhizal dependence (Hetrick et al., 1995). Similarly, there is substantial variation in maize lines for response to mycorrhizal fungi; however, mycorrhizal responsiveness appears to be related to soil P levels. In one study, all corn lines tested showed a positive response to AM fungi when grown under low P conditions (106 to 800% increase in shoot biomass), but mycorrhizal-infected corn grown in high P soils accumulated only 88% of the biomass of nonmycorrhizal corn grown in the same soil (Kaeppler et al., 2000). An alternative hypothesis to that of Hetrick et al. (1995) is that selective breeding practices have increased the inherent genetic ability of modern cultivars to take up nutrients without reliance on microorganisms.

41.4.3 Low-Input Systems

Low-input systems use mainly animal manures and leguminous plant residues (green manure) instead of chemical fertilizers to manage soil nutrient levels. The addition of organic C substrates benefits fungal biomass due to the lessening of competition with bacteria in a typically C-limited environment. Inputs of organic rather than inorganic nutrients to soil also have the potential to impact soil fungal communities and AM fungi, in particular, due to the sensitivity of many AM fungi to high levels of inorganic nutrients, especially P. In a long-term field study, percent root colonizations by AM fungi were 30 to 60% higher in vetch–rye, winter wheat, and grass–clover crops grown in soil from low-input farming systems than in crops grown in conventional systems (Mäder et al., 2000). Root colonization declined with increasing soluble P content, so differences in AM fungal colonization reflected partly the intensity of fertilizer input and soil P levels. In addition, the presence of pesticide residues in conventionally managed systems may have also inhibited root colonization by AM fungi. In a series of studies conducted at the Rodale Institute, the impact of low-input management on AM fungal spore populations and colonization potentials for different plant species were examined (Galvez et al., 1995, 2001a, 2001b). The low-input system relied on an overwintering cover crop, hairy vetch, or rye for nutrient management and pest control, whereas the conventional system used chemical fertilizers and pesticides; treatments were established 5 years prior to sampling. Winter cover cropping increased AM fungal spore populations and root colonization of cash crops, compared with the conventional treatment, presumably because the cover crop provided a host environment for AM fungal mycelia, which typically survive only 2 to 4 weeks once separated from a host (Galvez et al., 1995). Fertilizer use in the conventional plots may have also reduced spore populations and root colonizations (Galvez et al., 2001b). Enhanced AM fungal spore populations and root colonizations in low-input plots vs. conventional plots were associated with greater P and N use efficiencies in cash crops of maize and winter wheat, but only during early stages of wheat growth. However, enhanced AM fungal inoculum and P uptake did not translate into enhanced growth or yields of either maize or corn. Some explanations for the lack of yield response were lack of effective weed control, possibly insufficient N supply in the low-input system, or naturally high soil P levels that limited the benefits of AM fungal associations (Galvez et al., 2001a, 2001b).

Studies have also examined the impact of low-input practices on fungal community structure and diversity and have reported mixed results. For example, populations of *G. occultum* type spores were more abundant in a low-input agricultural soil, whereas conventionally farmed soil had greater populations of *G. etunicatum* type group spores (Douds et al., 1995). In another low-input field experiment, spores of *G. gigantea* were more numerous in low-input plots (up to 30 spores in 50 cm^3) than in conventional plots (<0.3 spores in 50 cm^3) (Douds et al., 1993). Enrichment of *G. gigantea* was possibly due to the decrease in chemical inputs and increase in vegetative diversity in the low-input systems, which had at least five different crop species, compared with only two species in the conventional treatment. While these studies provide evidence that conversion from conventional to low-input management can alter species composition of fungal communities, others have demonstrated that at least in some locations, conventional agricultural practices may maintain high indices of microbial diversity in the rhizosphere. Buyer and Kaufman (1996) examined the diversity of 40 soil rhizosphere fungal genera from long-term conventional and low-input treatments using culture-based methods. The major fungal taxa identified across all treatments were *Cladosporium*, *Fusarium*, *Gliocladium*, *Myrothecium*, *Penicillium*, *Trichoderma*, and *Verticillium*. No significant differences in fungal diversity were detected among treatments, but seasonal effects on fungal community structure were significant. A second study on the same plots also found no differences in AM fungal community spore structure and diversity, despite 15 years of management (Franke-Snyder et al., 2001). Similarly, AM fungal diversity was unaffected by low-input/organic rotations compared with conventional cereal systems after 17 years of management (Vestberg et al., 2002). Explanations for the lack of change in response to low input vary. In some experiments, elevated N and P status of soil and use of moldboard plow in all treatments may have homogenized community structure despite differences in crop diversity and form of nutrient management (Franke-Snyder et al., 2001). Interactions involving soil edaphic factors, such as soil P, organic matter, or pH, and other biotic factors, including presence of weeds, may also prevent clear-cut associations between diversity and management intensity (Kurle and Pfleger, 1996).

41.5 SUMMARY

This chapter describes important contributions of fungal communities in agroecosystems to soil conservation and plant productivity, mainly through their functional roles in aggregate formation and stabilization and nutrient cycling activities. There is no doubt that agricultural practices that disrupt the physical soil habitat and substrate resource availability impact fungal communities both structurally and functionally. However, conversion of agroecosystems from conventional- to alternative-based management can alleviate disturbance pressures to some extent, thus allowing for a change in fungal community biomass, activity, or structure to occur. Overall, discussions presented in this chapter are summarized as follows:

1. Conventional tillage practices have adverse effects on overall soil fungal biomass and hyphal lengths due to physical disruption of mycelial networks, redistribution of substrate resources, and degradation of soil aggregate structure. Changes in species composition have been detected within communities of AM fungi, with *Glomus* species predominating in tilled soils. As a result, species of *Glomus* have been classified as tillage resistant relative to non-*Glomus* AM fungi, including *Gigaspora*.

2. In monocropped systems, the presence of the same crop species over time exerts selective pressures on rhizosphere fungal communities, which become dominated by certain rhizosphere fungi and host-specific fungal symbionts and pathogens. Evidence suggests that within AM fungal communities, monocropping leads to the selection of aggressively colonizing and less mutualistic species that drain hosts of C and cause yield declines. Several species of *Glomus* have been identified as ineffective mutualists whose populations increase in response to monocropping. Fallowing also has detrimental impacts on fungal communities due to a reduction in C inputs to soil, as well as the absence of host roots for AM fungi.
3. Chemical fertilizer inputs can alter the ratio of active, cytoplasm-filled hyphae to total hyphae of fungi in soil. Fertilizers can also alter the species composition of fungal communities in general, as well as species composition of AM fungal communities. *Glomus* appears to be fertilizer insensitive, whereas populations of *Gigaspora* and *Scutellospora* generally decline in response to fertilizer inputs.
4. Alternative agronomic practices that seek to conserve soil resources have the potential to benefit fungal communities via cessation of physical disruption by no-tillage practices, increased plant species diversity, reduction of fallow frequency with crop rotations, and addition of C substrate resources by cover crop residues and animal manures. Such practices can increase levels of fungal biomass in soil, hyphal lengths, and AM fungal inoculum and root colonization potentials. Positive responses of soil fungi to alternative practices can lead to an increase in soil aggregation formation and stabilization and nutrient sequestration, as fungi are important contributors to these soil processes.
5. Within AM fungal communities, non-*Glomus* species are detected more frequently in alternatively managed agroecosystems than in systems under conventional management due to the alleviation of certain stresses. This shift in AM fungal community structure can have significant functional consequences in terms of soil conservation and mutualistic benefits to host plants. For example, *Gigaspora* appears to be more effective in stabilizing soil aggregates than *Glomus*, and hyphae of *Acaulospora* can spread faster and further in soil, and thus transport P over longer soil-root distances than hyphae of *Glomus*. A shift to more diverse species of AM fungi in alternative systems may allow for the development of better-optimized host–symbiont associations that permit maximum host benefits under a wide variety of environmental and cultural stresses.

More research into fungal community structure within agroecosystems is needed if we are to understand the importance of species diversity to ecosystem stability and resiliency to environmental perturbations. Studies conducted to date have shown that at least within AM fungal communities, species composition shifts can have important functional impacts and that the level of AM fungal diversity overall is important for maintaining plant community diversity and productivity (van der Heijden et al., 1998; Bever et al., 2001). As in many of the studies discussed here, AM fungal diversity is traditionally assessed by extracting spores from soil and identifying species according to spore morphologies. This approach can be highly misleading, however, as the relative abundance of a given spore type may not reflect the biomass or functional importance to the community (Douds and Millner, 1999). Instead, the abundance and diversity of mycorrhizal fungi must be assessed where the fungus is functionally relevant (i.e., in plant roots). Recently, molecular methods have been described that employ DNA primers to specifically target the 18S ribosomal DNA or the internal transcribed spacer region of AM fungi-colonizing plant roots (Simon et al., 1992; Helgason et al., 1998;

Renker et al., 2003). Targeted DNA can then be amplified by polymerase chain reaction (PCR) and analyzed to assess the genetic diversity or abundance of AM fungi *in planta* (Edwards et al., 1997). To date, molecular analysis of root-colonizing AM fungi has been conducted to study the natural symbionts of bluebell (Helgason et al., 1999) and to compare the diversity of AM fungi among roots of several grass species (Vandenkoornhuyse et al., 2002b, 2003) and between roots of woodland and arable plants (Helgason et al., 1998; Daniell et al., 2001). No studies have been published yet that have compared AM fungal diversity *in planta* between conventional and alternative cropping practices. It is vital that such work be conducted so that management-induced shifts in root-colonizing AM fungal communities can be linked to functional changes such as P uptake and translocation and mycorrhizal efficiencies.

Even less is known regarding shifts in species diversity of saprophytic fungi in response to disturbance, or whether saprophytic fungi are functionally redundant or operate within specific ecological niches. However, the use of fungal-specific primers is allowing a detailed examination of the genetic diversity of fungi in soils as well as plant roots. Recently, the molecular diversity of fungal communities has been examined in a pathogenic nematode-suppressive soil (Valinsky et al., 2002), in a wheat rhizosphere (Smit et al., 1999), and in the bulk and maize rhizosphere of a tropical soil (Gomes et al., 2003). Moreover, molecular-based studies are revealing an extraordinary, and unexpected, level of diversity within fungal communities of natural environments. For example, in what the authors considered a "mundane" habitat, fungal diversity in a single plant root system was quite extensive, with almost 50 different phylotypes, of which only 7 were closely similar (>99%) to known fungal sequences (Vandenkoornhuyse et al., 2002a). These studies give a glimpse of the biological potential that may be found in soils and plant roots of agroecosystems. The level of soil fungal diversity, its functional importance to ecosystem stability, and its response to various environmental perturbations represent unexplored but critical opportunities that warrant future research in order to advance the field of soil microbiology and soil science in general.

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42

Effects of Forest Management on Fungal Communities

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42.1 INTRODUCTION

Plant community composition, soil moisture, pH, and temperature, as well as the availability and quality of organic substrates and mineral nutrients, are key factors that control fungal communities and the processes they mediate (Keenan et al., 1993; Visser and Parkinson, 1999). These factors can be significantly altered by forest management practices (Frazer et al., 1990; Keenan et al., 1993); thus, these changes are likely responsible for transformations in fungal communities following disturbances. In this chapter, we address three major guilds of fungi: mycorrhizal fungi, saprotrophs, and pathogens. These three groups potentially respond differently to forest management practices, due to their different trophic attributes. For example, we would expect that clear-cut logging would have a greater negative impact on the mycorrhizal fungi colonizing roots of trees than it would on saprotrophs because the former rely predominantly on living trees for their carbon supply (Haselwandter et al., 1990; Smith and Read, 1997).

42.2 MYCORRHIZAL FUNGI

42.2.1 Introduction

Forest management can affect mycorrhizal fungi in several ways. First, and probably most important, mycorrhizal fungi will be affected by the removal of their hosts through

harvesting as well as by any resulting change in plant species composition in the remaining forest. Second, mycorrhizal mycelia may be disrupted by perturbations to the forest floor. Finally, changes in the amount of coarse woody debris will influence some mycorrhizal fungi.

All of the major types of mycorrhizal fungi are found in forests: ectomycorrhizal, arbuscular mycorrhizal, ericoid mycorrhizal, and orchid mycorrhizal; however, there is far more information on the effects of forest management on ectomycorrhizal fungi than on other types. Accordingly, most of our discussion will focus on this group. Most trees in the Betulaceae, Casuarinaceae, Corylaceae, Pinaceae, Fagaceae, Myrtaceae, and Tiliaceae are primarily ectomycorrhizal (Harley and Harley, 1987). This means that the major coniferous and eucalypt forests of the world are dominated by ectomycorrhizal fungi (although other types will be found associated with the understory). The Cupressaceae, Magnoliaceae, Oleaceae, Platanaceae, Taxaceae, and Ulmaceae tend to form arbuscular mycorrhizas, although some species will form ectomycorrhizas as well. Some members of the Aceraceae, Juglandaceae, Rosaceae, and Salicaceae primarily form ectomycorrhizas, while others form arbuscular mycorrhizas, or even both kinds (Harley and Harley, 1987). Hence, in mixed temperate deciduous forests, oak, beech, hornbeam, and linden trees are likely to be ectomycorrhizal, while elm, ash, maple, sweet gum, and sycamore, among others, are likely to be arbuscular mycorrhizal. The majority of tropical forest trees are arbuscular mycorrhizal (Onguene and Kuyper, 2001; Zhao et al., 2001, 2003), but there are important tropical trees that are ectomycorrhizal, including some members of the Myrtaceae, Dipterocarpaceae, and Fabaceae (Alexander et al., 1992; Lee et al., 1996).

42.2.2 Effects of Forest Management on Ectomycorrhizal Fungi

42.2.2.1 Tree Removal

The most extreme case of forest management is clear-cut harvesting or clear-felling, where the stems of all but the smallest trees are removed. Occasionally trees from species that are not of commercial interest are left standing. After clear-cut logging, new stands can regenerate naturally or the sites can be planted with seedlings produced in nurseries. Among selected countries with primarily ectomycorrhizal forests, clear-cutting accounts for 20% (Scandanavian countries and the state of Victoria in Australia) to 90% (Canada) of the harvested area (Jones et al., 2003).

To understand the effect of clear-cut logging on soil organisms, it is useful to compare it with more natural disturbances. The same temperate forests that are harvested by clear-cutting tend to be subjected to wildfire under natural conditions. Wildfires can kill trees in large areas, and thus it has been claimed that clear-cutting mimics fire. Nevertheless, there are several important differences (Bulmer et al., 1998; Giardina and Rhoades, 2001). First, stand-destroying fires can eliminate the upper, organic soil layers. This releases carbon to the atmosphere, but often leaves behind ash, which contains nutrients in forms that are readily available to plant roots. The forest floor can be disturbed during clear-cut logging or in subsequent site preparation treatments, but the resulting chemical changes are slower than with fire. For example, mineralization of nitrogen often increases after clear-cutting, and this results in an increase in the levels of extractable inorganic nitrogen levels, referred to as the assart flush, but this tends to occur several years after clear-cut logging (Prescott, 1997; Prescott et al., 2003). The pH of the upper horizons increases after fire, but not after clear-cutting. Second, wildfires vary in intensity and can sometimes just burn the understory and part of the forest floor, without killing the trees, whereas clear-cutting removes all commercially important trees. Still, at the landscape level both types of disturbances can result in fragmented forest cover. Thus, although there are

significant differences between the effects of wildfire and clear-cutting, the spatial distribution of the two disturbances may be quite similar.

Following clear-cutting, ectomycorrhizas associated with roots from the previous stand die over 1 to 3 years (Harvey et al., 1980b; Hagerman et al., 1999a), depending on the site. Mycorrhizas formed by different fungi seem to persist for different lengths of time (Harvey et al., 1980b; Hagerman et al., 1999a), but all eventually die as the root systems die. Nevertheless, ectomycorrhizal fungi persist in the soil as asexual and sexual spores or as sclerotia (Miller et al., 1994). A recent reexamination of ectomycorrhizal colonization of seedlings regenerating after clear-cutting concluded that seedlings planted in clear-cuts are as heavily colonized as those planted in forests (Jones et al., 2003). The persistence of these other forms of inoculum, or dispersal of additional spores to the site, is presumably responsible for this (Fox, 1983, 1986; Bâ et al., 1991). One other potential source of ectomycorrhizal fungal inoculum on clear-cuts is the roots of so-called refuge plants (Kranabetter, 1999; Hagerman et al., 2001). Any ectomycorrhizal or arbutoid mycorrhizal (arbutoid mycorrhizas are formed by the same fungi as ectomycorrhizas) shrubs or trees left behind after clear-cutting can be considered to be refuge plants, although the complement of mycorrhizal fungi on some refuge species will match those of the new post-clear-cut host better than others (Kranabetter, 1999; Hagerman et al., 2001). Refuge plants may be important because some ectomycorrhizal fungi appear to be able to colonize roots only from hyphae attached to living roots of other trees (Fleming, 1984; Simard et al., 1997; Kranabetter et al., 1999). This may be the reason that some studies have found the highest levels of colonization or ectomycorrhizal fungal diversity around the periphery of clear-cuts, within the rooting zone of forest trees (Kranabetter and Wylie, 1998; Durall et al., 1999; Hagerman et al., 1999b).

Rather than a reduction in colonization after clear-cutting, the major impact on ectomycorrhizal fungi is a change in species composition of the ectomycorrhizal fungal community (Jones et al., 2003). This is not surprising given that so many factors are changed as a result of clear-cutting: the age and species composition of potential plant hosts; the biological, chemical, and physical properties of the soil; and introduction of ectomycorrhizal fungi along with any planted seedlings. In general, fungi such as *Wilcoxina* spp. and *Thelephora* spp., which can disperse effectively via airborne sexual spores, increase in relative abundance in stands regenerating after clear-cutting (Kranabetter and Wylie, 1998; Hagerman et al., 1999b; Mah et al., 2001). Jones et al. (2003) concluded that both changes in the environment associated with clear-cutting and loss of inoculum were important in causing species shifts in the ectomycorrhizal fungal community. We await further research to determine whether the fungi that dominate young stands regenerating after clear-cutting are better adapted to absorb and translocate nutrients from these soils or whether they are dominant because they are more competitive colonizers of root systems.

42.2.2.2 Partial Cutting or Thinning

Very little work has been done on the effects of partial cutting or selective logging on mycorrhizal status of the remaining trees. In one of the few studies to investigate this, DeBellis et al. (2002) found no change in the percentage of roots colonized or the composition of the fungal community associated with *Betula alleghaniensis* growing in gaps created by partial cutting. We would not predict a major effect on colonization unless all members of one tree species were removed from a stand (see below).

42.2.2.3 Changes in Tree Species

When logged sites are restocked with nursery seedlings, a different tree species may be planted from those in the original stand. Tree species that grow more rapidly or that are

more commercially valuable than the original tree species on a particular site may be selected. Frequently, this results in a loss of tree diversity compared with the previously existing stand. What are the likely effects of reducing the diversity of trees on the mycorrhizal fungal community? In the few studies to address this question, there is some suggestion that fungal diversity is lower in stands of single tree species than in mixed stands. For example, when Douglas fir was grown together with paper birch, the evenness of the ectomycorrhizal fungal community on its root system increased, at least temporarily, compared with when it was grown separately (Jones et al., 1997). The reason for the increased diversity in this case may be because some fungi will only associate with a tree species (referred to as a secondary host) if it is already associated with another tree species (the primary host). High tree diversity may also result in higher species richness of ectomycorrhizal fungi on a site. A recent study of mixed deciduous–coniferous stands in eastern Canada (Kernaghan et al., 2003) found a clear positive relationship between the diversity of trees in the overstory and the diversity of ectomycorrhiza morphotypes present in the soil. In this study, the authors speculate that increased fungal diversity was associated with a more diverse leaf litter and, therefore, an increased range of substrates for the fungi to colonize. It is important to remember that ectomycorrhizal fungi are closely related to saprotrophic fungi (Hibbett et al., 2000). Consequently, they receive most of their mineral nutrients and some of their carbon by secreting enzymes that mineralize organic substrates in litter (Bending and Read, 1995a, 1995b). If some tree species present in the original stand are excluded from the regenerating stand, then some ectomycorrhizal fungi would be expected to disappear from the community. Many ectomycorrhizal fungi have broad host ranges; i.e., they can form mycorrhizas on many different tree and shrub species; however, others will only associate with specific tree species (Molina et al., 1992). Because ectomycorrhizal fungi cannot survive in an active form for long periods without energy supplied by the host tree, we can predict that fungi with high host specificities will disappear from a site over the long term unless their tree host is present.

42.2.2.4 Site Preparation

If a site is to be replanted with nursery-grown seedlings, it is common to prepare the site, either manually or mechanically. In cold or wet sites, the upper 30 cm or more of soil is frequently dug up and inverted to create planting sites in the shape of mounds or ridges. These raised microsites have higher soil temperatures (Sutton, 1993; Bedford et al., 2000) and are better drained (de Chantal, 2004) than unprepared sites. Another common practice is scalping or screefing, where the upper, organic soil layers are pushed aside in small microsites or over larger areas, to expose the mineral soil. Removal of the forest floor allows the mineral soil to heat more rapidly (Simard et al., 2003). In the past, broadcast burning was widely used after clear-cutting to release nutrients and expose the mineral soil. It is less commonly used now because of concerns about air quality.

Inoculum of ectomycorrhizal fungi, whether in the form of ectomycorrhizas, hyphae, spores, or sclerotia, is most common in the upper few centimeters of forest soils (Harvey et al., 1986; Brundrett et al., 1996). Ploughing, mounding, and screefing will therefore disrupt or displace ectomycorrhizal inocula. Although colonization rates and ectomycorrhizal fungal diversity of seedlings planted in screefed sites can be reduced initially (Simard et al., 2003), this does not always happen (Dahlberg, 1990; Baar and de Vries, 1995). When it does occur, it does not appear to be a long-term effect (Simard et al., 2003), and it disappears as the roots extend beyond the screefed patch.

Burning, on the other hand, can cause loss of substantial levels of inoculum (Harvey et al., 1980b; Stendell et al., 1999) and long-term reductions in colonization, especially

when the fire is hot enough to eliminate the humus (Mikola et al., 1964). In other cases, burning after clear-cutting does not affect percent colonization of roots (Herr et al., 1994). The composition of the fungal community forming mycorrhizas often changes after broadcast burning or wildfire (Torres and Honrubia, 1997; Grogan et al., 2000; Mah et al., 2001), suggesting that some fungi survive fire better or are more competitive colonizers in the changed soil conditions.

42.2.2.5 Coarse Woody Debris

Commercial forestry may result in second-growth stands with less coarse woody debris than natural stands (Graham et al., 1994). This would be expected to reduce fruiting of fungi, such as *Piloderma* spp., *Amphinema* spp., *Tomentella* spp., and sebacinoid fungi, which typically fruit on decaying logs. It might also affect the species composition of the ectomycorrhizal fungal community because some fungal species, such as *Piloderma fallax*, are more common in rotting wood than in other substrates (Goodman and Trofymow, 1998; Mah et al., 2001). Several studies have concluded that decomposed wood forms "hot spots" of ectomycorrhizal fungal inoculum (Kropp, 1982; Väre, 1989; Harvey et al., 1997); therefore, reduction in coarse woody debris may result in a reduction in ectomycorrhizal fungal diversity in the long term.

42.2.3 Effects of Forest Management on Sporocarps of Ectomycorrhizal Fungi

For many years, the presence of sporocarps of ectomycorrhizal fungi was considered to be indicative of the belowground ectomycorrhizal fungal community, but it is now well established that the relative abundance of mushrooms of different species is not well correlated with the fungal community found on the root systems (Dahlberg et al., 1997). This is due, in part, to the dominance of some forests by ectomycorrhizal fungi from the Thelephoraceae and Ascomycota, which do not form conspicuous fruit bodies (Horton and Bruns, 2001). Nevertheless, sporocarps are important in their own right, both as sexual reproductive structures and as food sources for small mammals and insects (Wiensczyk et al., 2002).

Forestry practices can affect the abundance of fruit bodies produced by ectomycorrhizal fungi. For example, several studies have found a positive correlation between the number of ectomycorrhizal host species and the number of ectomycorrhizal fungi that are fruiting (Ferris et al., 2000; Kranabetter, 2001). This suggests that planting mixtures of tree species would increase the range of ectomycorrhizal fungi that would fruit in a second-growth stand. Furthermore, Durall et al. (1999) found a clear threshold in gap size, above which the number of mycorrhizal fruit bodies decreased. In gaps larger than 900 m^2, the number of species fruiting dropped by 87%. Production of hypogeous sporocarps (truffles) stopped altogether in clear-cut gaps of 0.1 ha or larger in a subalpine forest of *Abies lasiocarpa* and *Picea engelmannii* (Durall, unpublished) during the first 5 years after harvest, and this major reduction in fruiting appears to last up to 30 years (Amaranthus et al., 1994). In some studies, selective harvest or thinning appears to have little clear overall effect on production of epigeous or hypogeous ectomycorrhizal sporocarps, although the frequency of occurrence of individual species may be affected (Kranabetter, 2001). However, 50% thinning of a *Pinus sylvestris* forest seemed to initiate a succession in fruit body production, with the production of certain species peaking over several years and then decreasing to minor components of the vegetation (Shaw et al., 2003).

42.2.4 Tropical Forests and AM Fungal Inoculum

Large areas of wet tropical forest are being cleared and converted to pasture. Although the vast majority of tropical trees form arbuscular mycorrhizas (Alexander et al., 1992), the same type of mycorrhizas as pasture grasses, there has still been concern that a reduction or change in inoculum of arbuscular mycorrhizal fungi could prevent regeneration of forests on these sites. Almost all studies comparing the propagule loading and diversity of arbuscular mycorrhizal inoculum have found the same or higher levels in tropical pastures as in adjacent forests (Asbjornsen and Montagnini, 1994; Fischer et al., 1994; Maldonado et al., 2000; Picone, 2000) and concluded that mycorrhizal inoculum does not limit tree reestablishment. An exception was Allen et al. (1998), who found similar inoculum potential but reduced fungal richness in pastures when compared with adjacent forests. The fires that accompany forest clearing may act independently to reduce the inoculum potential of the soil, but this appears to be a short-term effect providing that vegetation reestablishes quickly (Bellgard et al., 1994; Rashid et al., 1997). Another series of studies has found that inoculum levels do not decrease in tropical forest gaps, such as those formed by natural disturbance or selective logging (Guadarrama and Alvarez-Sanchez, 1999; Whitbeck, 2001; DeBellis et al., 2002). Some types of tropical forest regenerate to vegetation types that are not dominated by arbuscular mycorrhizal plants, once the sites have been cleared. In some cases, this has caused a reduction in the inoculum potential of arbuscular mycorrhizal fungi (Hopkins et al., 1996), but in others, it has not (Allsopp and Holmes, 2001).

42.3 SAPROTROPHIC FUNGI

42.3.1 Introduction

The saprotrophic fungi, which are major contributors to organic matter decomposition, are ubiquitous throughout the forest. For example, they are present in the aerobic layers of the mineral soil, soil organic layers, litter layers, and decaying wood and on all surfaces of living plants. In this section we will emphasize the response of saprotrophic fungi located in the organic and mineral layers of the soil to several forest management practices.

42.3.2 Methodology

Various methods have been used to describe the community structure of saprotrophic soil fungi with respect to forest management practices. All of these methods have shortcomings, and it is important that researchers be aware of these. Traditional taxonomic methods involve isolating fungi from washed particles (Parkinson and Thomas, 1965) or from serial dilutions (Wicklow and Whittingham, 1978) and identifying fungi by observing anamorphic or teleomorphic structures. This method can be very time consuming, and it selects against fungi that cannot grow in culture or those that are less competitive than fast-growing saprotrophs (Parkinson and Coleman, 1991). Nevertheless, it can be used in a way that describes specifically the fungal saprotrophs and not other fungal trophic groups, such as the mycorrhizal fungi. Chemical techniques, such as the use of signature phospholipid fatty acids (PLFAs), have been useful for estimating the fungal-to-bacterial ratio in the microbial biomass following clear-cutting (Bååth et al., 1995; Siira-Pietikäinen et al., 2001). This technique has also detected species changes in the microbial community, which can occur following fire treatment, clear-cutting, and liming (Frostegård et al., 1993; Bååth et al., 1995). This method does not distinguish between saprotrophs and ectomycorrhizal fungi, but it will be able to detect organisms that cannot be cultured. DNA-based techniques, such as denaturing gradient gel electrophoresis (DGGE), in combination with

specific primers targeting fungal rDNA (Muyzer et al., 1993; Kottke, 1994; Kowalchuk et al., 1997; Chen and Cairney, 2002), nested PCR (Van Tuinen et al., 1998), or the combination of these techniques with competitive PCR, can distinguish between species and also quantify their relative abundance (Kowalchuk, 1999). As with the PLFA method, these techniques can detect organisms that will not grow in pure culture. However, in order to identify fungi using DNA methods, a database of known fungi must be available for comparison. Furthermore, the extraction and amplification of DNA from soil samples can be problematic. These are clearly drawbacks of DNA-based techniques. Thus, it may be desirable to combine conventional taxonomic methods with new DNA-based methods to achieve a more accurate characterization and quantification of the fungal community (Kowalchuk, 1999).

42.3.3 Effects of Forest Management on Saprotrophic Fungi

42.3.3.1 Tree Removal

Few studies have investigated the effect of clear-cut harvesting and subsequent site preparation on the community structure of saprotrophic fungi. Overall, minimal effects of clear-cutting on decomposer community structure and on the processes linked to these organisms have been reported. In addition, the effects that have been found are substantially less severe than those imposed by wildfire (Wicklow and Whitingham, 1978; Bååth et al., 1995; Visser and Parkinson, 1999), with either no (Houston et al., 1998a; Visser and Parkinson, 1999) or minimal (Wicklow and Whittingham, 1978; Bååth, 1981; Houston et al., 1998a) effects on saprotrophic community structure. Effects of clear-cutting or wildfire have been largely attributed to the quantity and quality of the organic matter substrate. The deviation in results between different studies is due not only to differences in site characteristics and soil location, but also to the specific factors that were measured. For example, Houston et al. (1998a) found no effects of clear-cutting when based on rank abundance curves, but a significant effect when species richness and species diversity were calculated. They found that diversity and species richness of fungal decomposers decreased in the organic layer, whereas it increased in the mineral layer in response to clear-cutting. The authors suggest that increased diversity in the mineral layer may be due to a response to an intermediate disturbance.

Increases in diversity due to intermediate disturbances have been reported for other organisms (Connell, 1978). The idea is that disturbances, which do not impact all organisms present in an area, may create new habitat conditions, allowing an additional set of organisms to colonize. In the case of the Houston et al. study, clear-cutting was apparently disruptive enough to reduce fungal decomposer diversity and species richness in the organic soil layer, but not enough to do so in the mineral soil layer. Significant differences between clear-cut and control soil were not evident for basal respiration, microbial biomass C, microbial C/organic C, and nitrogen mineralization. Thus, the relationship between organic and mineral soil layers did not translate over to microbial processes and metabolic quotients. This is supported by the idea that functional redundancy, where different species of organisms perform the same function, reduces the importance of species diversity with respect to overall ecosystem functioning (Gitay et al., 1996). Some studies have found differences between clear-cutting and control sites in PLFA patterns and in fungal-to-bacterial PLFA ratios (Bååth et al., 1995; Siira-Pietikäinen et al., 2001), but much of this difference would likely be attributed to a decrease in active ectomycorrhizal roots (Bååth et al., 1995). As previously discussed, root systems quickly lose their ability to support ectomycorrhizae after clear-cutting practices (Harvey et al., 1980a).

The long-term temporal change of the soil fungal community following clear-cutting is minimal compared with long-term effects of site fertility on the soil fungal community.

For example, Pennanen et al. (1999), using PLFA analysis in Finland, found that fertility of the site affected the fungal structure of the coniferous forest humus more than the successional stage of the forest.

42.3.3.2 Partial Cutting, Thinning, and Site Preparation

Although minimal effects of clear-cutting have been found on the community structure of saprotrophic fungi, the effects (based on PLFA analysis) of alternative harvesting methods, such as partial felling (30% of the stand removed), and the effects of site preparation (e.g., shallow trenching or harrowing) are either absent or tend to be less than the effects of clear-cutting alone (Siira-Pietikäinen et al., 2001).

42.3.3.3 Site Preparation

Site preparation, also referred to as vegetation management, can be performed using chemical (herbicide such as glyphosate) or mechanical (e.g., brush saw) methods. As stated above, it has been shown to decrease competition between growing conifers and noconiferous vegetation (Ehrentraut and Branter, 1990). In British Columbia, this technique is often performed on sites 4 to 10 years after clear-cutting. Relatively few studies have followed the effects of vegetation management on the soil microbial community and on the processes they mediate (Ohtonen et al., 1992; Wardle and Parkinson, 1992), and only one that we know of has looked at its effect on fungal community structure in the field under normal operational conditions (Houston et al., 1998b). Herbicides have been shown to indirectly affect the soil microbial biomass through direct effects on soil moisture, temperature, and organic substrate quantity and type (Wardle and Parkinson, 1990). Some studies have found effects on microbial biomass with high doses of herbicide administered in the laboratory but not in the field (Olson and Lindwall, 1991). These results are supported by the findings of other field studies, which have used operational levels of herbicides and have found minimal effects on microbial biomass and microbial processes (Ohtonen et al., 1992; Houston et al., 1998b). In a field study conducted in Ontario, Houston et al. (1998b), using two realistic levels of herbicide treatments, found few effects except that both herbicides reduced the isolation frequency of *Mortierella vinacea* in clear-cuts and in site-prepared organic soils. In addition, the brush-saw treatment reduced the isolation frequency of *Paecilomyces carneus* in clear-cut and prepared organic soil. Some rare species may have been impacted as well, but these could not be determined because isolation frequencies were too low for statistical analysis.

42.3.3.4 Changes in Tree Species

Another forest management practice that has high potential, in the long term, to affect the community structure of the saprotrophic fungi is the planting of seedlings in mixtures rather than in monoculture (Grayston et al., 1998). There are indications that the quality of soil organic matter as well as soil nutrient composition are factors responsible for the shifts in the microfungal community structure when sites differing in their plant composition are compared (McLean and Huhta, 2002). Although shifts due to clear-cutting in the saprotrophic fungal community have been minor, these changes may be important in terms of the spread of pathogenic fungi. For example, microfungal community structure differences and increases of fungal species that stimulate rhizomorph formation of *Armillaria ostoyae* and *Armillaria gallica* have been observed when comparing rhizospheres of live trees and stumps (Kwasna, 2001, 2003).

42.4 PATHOGENIC FUNGI

42.4.1 Introduction

Trees are usually the dominant vegetation feature of forests and are the objective of forest management. Therefore, most of what is known about pathogenic fungal communities in forests is limited to pathogens of trees. Very little is known about pathogens of other vegetative components of forest ecosystems.

Disease-causing fungi have frustrated forest management objectives by causing mortality and reductions in growth and wood quality of commercial tree species. Reductions in volume and economic value of wood products and difficulties with regeneration of forests result from pathogenic infections. However, the interaction between pathogens and forest management is not unidirectional. Forest management practices can have significant effects on pathogenic fungal populations and communities, frequently with negative impacts on the goals and objectives of the forest management practice.

Fungal pathogens of trees are found primarily in the Basidiomycota and Ascomycota although there are some in the Fungi Imperfecti and in the Oomycota.* Two subclasses of the Basidiomycota are of particular importance with forest pathogens. The hymenomycetes include fungi that cause wood decays and root rot. The teliomycetes cause rust diseases of bark, foliage, and cones. The Ascomycota include fungi that cause foliar diseases and stem cankers.

Pathogenic fungi are natural components of forest ecosystems. Many fungi, although pathogenic on their tree host, contribute to ecosystem function by providing weakened wood for cavity habitat, creating openings and therefore structural diversity in the canopy, and enabling the breakdown of dead wood. The selection pressure placed on host trees by a pathogen has resulted in resistance mechanisms and other adaptations that limit the spread of the pathogen and the damage that it causes. Consequently, native pathogens and their hosts tend to be in balance with each other, with neither one gaining the upper hand. These natural components of forested ecosystems become pests when the pathogen interferes with some human-imposed management objective. Therefore, the concept of pest is dependent upon perspective.

There are three ways that pathogens become pests:

1. When nonnative pathogens, or hosts, are introduced to a new environment where the host and pathogen have not evolved together, they can be considered pests. Resistance mechanisms are nonexistent in the host, and if the introduction is successful, the pathogen can dominate the host–pathogen relationship. There are numerous examples of devastation caused by the introduction of forest pathogens. White pine blister rust was introduced in North America at various times between 1898 and 1910 on white pine nursery stock (Detwiler, 1928) and has effectively eliminated five-needle pines as commercial species throughout much of the U.S. and Canada. Likewise, chestnut blight has obliterated the American chestnut from forests that it once dominated. It is thought that both of these organisms originated in Asia where native species show some resistance (Bingham, 1983; Roane et al., 1986).
2. Natural levels of forest pathogens can be viewed as pests when the land base available for forest management shrinks and more pressure is placed on individual stands to maximize yield. The amount of damage caused by the pathogen

* The oomycetes are now considered by many taxonomists to be in the Kingdom Stramenopila (fungal and algal groups with cellulose in cell walls).

may remain the same, but the significance of the damage changes as more demand for production is placed on a smaller land base. For example, in the early days of forest harvesting, decay in the butt of logs was not considered important and was just cut off and left behind. Forests were abundant, and the pressure on any particular stand to produce high yields was low. Today, past harvesting, urbanization, and protected areas have reduced the available timber harvesting land base, and consequently, losses due to decay are less acceptable even though the amount of decay may not have changed. This change in perception has resulted in shorter rotations in some managed forests to reduce the damage caused by decay fungi.

3. Native forest pathogens operating at low levels in natural ecosystems may become pests as a result of specific forest management practices that disrupt the balance between host and pathogen. The remainder of this section focuses on the disruption of the balance between host and pathogen management practices.

42.4.2 Disease Triangle

Disease, whether it occurs in trees or in people, requires three elements that are commonly referred to as the points of the disease triangle: a pathogen, a susceptible host, and appropriate environmental conditions. All three elements must coincide to cause disease, and if one part of the triangle is altered in some way, there may be a corresponding effect on the presence or magnitude of disease (Tainter and Baker, 1996; Agrios, 1997).

Native hosts and pathogens have evolved together in specific environments, such that the development of resistance in the host and virulence in the pathogen have taken place in the context of a particular set of environmental circumstances and processes (Day, 1974). Forest management practices alter the environment and the ecosystem processes within it and, therefore, can alter the relationship between the three points on the disease triangle.

Traditional forest management involves establishment and tending of an even-aged crop of commercial tree species, following harvest of the previous stand. This often results in a different species composition, stand structure, and successional processes than what would exist following natural disturbance events such as fire. Species diversity is often reduced, and the prevalence of commercial species is increased across the landscape. Native pathogens of these species, previously limited by host availability, can increase rapidly in managed forests. This has been observed with several disease–host systems, for example, black-stain root disease (*Leptographium wageneri*) of Douglas fir in the western U.S. (Hansen et al., 1983) and red-band needle blight (*Dothistroma septospora* (= *D. pini*)) of lodgepole pine in north-central British Columbia (Woods, 2003).

The use of nonnative commercial species in plantations can further swing the balance in favor of the pathogen because of the lack of genetic resistance or vigor in the host or because the host is in a more favorable environment for disease development. In New Zealand, over 90% of commercial forests are planted with *Pinus radiata*, a nonnative species. It is uncertain when *D. pini* was introduced to New Zealand, but by 1966 it was widespread throughout the country where spore dispersal is favored by the moist, mild climate (Gibson, 1972). In the southeast U.S., fast-growing and commercially desirable *Pinus taeda* (loblolly pine) and *Pinus elliotti* (slash pine) have been planted extensively in areas previously occupied by *Pinus palustris* (longleaf pine) and *Pinus echinata* (shortleaf pine). Fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*) is native to the area, but its incidence has increased substantially due to this shift away from the genetically resistant species to more susceptible species (Powers et al., 1981).

42.4.3 Effects of Forest Management on Pathogenic Fungi

Tree pathogens form functional groups characterized by the part of the tree affected and by their mode of infection and spread. These groups are affected differently by forest management, and examples of each are discussed below.

42.4.3.1 Root Decay Fungi

Root decay fungi infect trees through the root system. Most have a parasitic, infectious stage and a saprotrophic stage that can persist from one generation to the next and lead to buildup of inoculum. Some root disease fungi, normally kept in check by temporal and spatial diversity in host availability and by defense responses in the host, have increased significantly as a result of forest management. Two examples follow.

Armillaria ostoyae causes root disease of conifers in the southern one third of British Columbia (Morrison et al., 1992). In moist, productive ecosytems, a majority of mature trees will have lesions caused by *A. ostoyae* on their roots, but will not exhibit symptoms of disease, presumably because trees on productive sites are vigorous enough to resist disease progression (Morrison et al., 2000). However, even on these productive sites, when infected trees are harvested for commercial or thinning purposes, the dynamic defense responses are lost, the balance between host and pathogen swings in favor of the pathogen, and it can then spread throughout the stump's root system. The amount of inoculum available to cause new infections increases significantly, placing residual trees at greater risk of infection. For example, Cruikshank et al. (1997) determined that mortality of crop trees caused by *A. ostoyae* occurred following precommercial thinning and further that the amount of mortality was greater in the interior Douglas fir and interior cedar hemlock forests than in coastal forests where trees had a greater ability to resist infection.

Levels of resistance to *A. ostoyae* also vary with host species. *Pseudotsuga menziesii* (Douglas fir) is one of the most susceptible species, while hardwoods such as birch and aspen are resistant (Morrison et al., 1992). Throughout the southern interior of British Columbia, Douglas fir has been heavily favored for regeneration of harvested areas due to its commercial value. This has resulted in a change in species composition on sites where the spread of *Armillaria* was previously limited by a diverse species composition, including species with tolerance or resistance to *Armillaria* (Hagle and Shaw, 1991).

Finally, in order to meet requirements to produce second-growth forests that are free of competition from shrubs and broad-leaved trees, manual weeding or applications of herbicides are carried out to remove species that may compete with coniferous crop trees. This practice affects inoculum levels of *Armillaria* by enabling a flush of inoculum in the stumps of cut broadleaf species that were colonized by *Armillaria* prior to treatment (Morrison and Mallett, 1996). Furthermore, there is evidence to suggest that the presence of broadleaf species has a direct, negative influence on the incidence of infection in the more susceptible conifer species (Morrison et al., 1988; Gerlach et al., 1997), although the mechanisms are unknown.

Heterobasidion annosum is a root decay fungus that attacks a number of conifer species in the northern hemisphere. The fungus can spread from stumps of previously infected trees, and from stumps infected by basidiospores at the time of felling, to surrounding regeneration (Rishbeth, 1951, 1957; Stenlid, 1987). Basidiospores can only infect freshly exposed wood surfaces (wounds or stumps) or stressed roots where the bark can be degraded and penetrated (Gibbs, 1967). The mycelium from colonized stumps can directly infect healthy trees across root contacts or grafts (Korhonen and Stenlid, 1998). Spread of the fungus in dead or dying roots is much faster than in roots of living trees (Rishbeth, 1951) but can be slowed by colonization by competitive saprotrophic fungi.

Regardless of the source of infection (previously colonized or infected by basidiospores following cutting), inoculum in stumps is a key factor in the spread of *H. annosum*. Intensive plantation management, including short rotations and thinning, is common throughout Scandinavia and northern European countries (Bendz-Hellgren et al., 1998; Dimitri and Tomiczek, 1998). This has resulted in increased opportunity for spore infections of cut stumps and wounded trees and for increased spread to surrounding trees. Bendz-Hellgren and Stenlid (1998) found that stumps created by commercial thinning operations were more susceptible to infection than clear-cut stumps or precommercially thinned stumps despite the similarity in sapwood area in clear-cut and thinned stumps. They suggest that availability of inoculum, differences in airflow dynamics, or stump moisture content could explain these observations.

42.4.3.2 Stem Decay Fungi

Stem decay fungi enter live trees either through natural openings (true heartrots) or through wounds. Living sapwood is very resistant to decay (Rayner and Boddy, 1988), so heartrot fungi have specialized mechanisms to bypass the sapwood in order to colonize the heartwood, where they can create extensive decay columns. Forest management can result in reductions in decay fungi through reduced harvest ages and increases in decay incidence due to wounds created during thinning or partial cut harvest operations.

Pathological rotation age is the age where annual growth increment no longer exceeds annual loss of wood volume due to decay (Manion, 1981). In species that are particularly susceptible to fungi that cause true heartrots, pathological rotation age often precedes natural rotation age (culmination of mean annual increment). Therefore, when the objective is to maximize timber volumes, commercial species are often harvested before extensive decay columns develop. This practice can lead to a reduction in older, decayed trees and, therefore, a reduction in potential cavity habitat. Because living sapwood is very resistant to decay (Rayner and Boddy, 1988) but dead sapwood is very susceptible to decay, tree hollows with sound outer cores can only be initiated by heartrot fungi that attack a living tree (Bull et al., 1997). The presence of stem decays in heartwood of living trees may be essential for primary cavity excavators to create cavity habitat (McClelland and Frissell, 1975), and the early harvest of trees can lead to a reduction of existing habitat and potential habitat recruits.

Some broadleaf species, such as trembling aspen, are particularly susceptible to stem decays. *Phellinus tremulae* causes a true heartrot in aspen and is thought to be the most economically important decay pathogen of aspen (Peterson and Peterson, 1992; Hunt and Etheridge, 1995; Callan 1998). Parsons et al. (2003) linked the presence of *P. tremulae* with maternal roosts of bats and suggested that the presence of *P. tremulae* is important to the stability of forest bat populations.

In locations where aspen is not considered a commercial species, the stocking level of aspen is often restricted (e.g., Ministry of Forests, 2000). Where aspen is considered a commercial species, its use has been increasing (Darrah, 1991), and forest management practices have focused on maximizing timber production through short-rotation management to reduce the volume lost to stem decay (Peterson and Peterson, 1995). Collectively, this may contribute to a reduced incidence of decay fungi in mature to old trees, and a reduction in the availability of suitable trees for cavity nests.

Forestry practices also provide new infection courts (wounds, adjacent stump surfaces) for wound-entry heartrot fungi (Stenlid, 1987; Whitney, 1991). Whitney (1991) found that in the 10 years following a thinning operation, 40% of resultant wounds had advanced decay and 90% had associated stained wood. Wound types most commonly

associated with decay included basal scrapes by logging equipment and broken tops. Thin bark species, such as spruce, are particularly susceptible to wound-entry decay fungi (Vasiliauskas and Stenlid, 1998b), and some plantations that are uniform in species, size, and spacing are susceptible to bark-peeling wounds from deer and moose, and subsequent infection by decay fungi (Vasiliauskas and Stenlid, 1998a).

42.4.3.3 Stem Rust Fungi

Stem rusts are caused by fungi in the subclass Teliomycetes and are unique in that most rust fungi require two botanically unrelated hosts to complete their life cycle, which consists of up to five spore stages. Rust fungi are obligate parasites, in that they must have a live host, and are host specific.

In general, highly managed plantations have resulted in intensification of stem rusts due to the high susceptibility of vigorously growing shoots and host uniformity. This is particularly the case with the autoecious *Endocronartium* or *Peridermium* rusts, which have no alternate host, and spores produced on the pine host can directly infect other pine trees (Hiratsuka, 1997).

Studies of the effects of thinning on rust incidence have shown varied results. Hills et al. (1994) observed an increasing infection incidence of western gall rust (*Endocronartium harknessii*) with decreasing stand density to a spacing of 1.5 m; then incidence decreased with wider spacings. Blenis and Duncan (1997) also found higher incidence of rust after thinning in areas that experienced a wave year* of infection. However, Blenis and Bernier (1986) found that forest opening size had no impact on western gall rust infection frequency. Likewise, Kaitera (2002) determined that thinning of Scots pine did not have an impact on incidence of stem rust (*Cronartium flaccidum*), although in this study the thinning operation included sanitation (removal of infected stems). Belanger et al. (1991) observed a decrease in fusiform rust sporulation on pine after sanitation thinning. The impact of thinning on rust fungi is highly variable and appears to depend on the species of rust fungus, prevalence and proximity of the alternate host, stem density, and degree of sanitation achieved by the thinning.

42.4.3.4 Wilt and Stain Fungi

Fungi that cause vascular wilt diseases and sap stains tend to be in the *Ceratocystis* and *Ophiostoma* genera. They are unique in that insect vectors play an important role in epidemiology. These fungi do not break down the wood cell structure, as do the decay fungi, but cause dysfunction in translocation due to the presence of hyphae in conducting tissues. The ophiostomatoid fungi have similar sexual states, but differ markedly in the asexual forms produced. This variety of spore forms and dispersal mechanisms makes the wilt fungi very adaptable, as seen by the elm pandemic caused first by *Ophiostoma ulmi*, followed by *Ophiostoma novo-ulmi* (Wingfield et al., 1993).

Stain and wilt fungi vary in pathogenicity and virulence. In some cases, only severely stressed trees are susceptible to infection, and in others, the fungus is capable of defeating the resistance mechanisms of a healthy tree (Gibbs, 1993). Black-stain root disease, caused by *Ceratocystis wageneri* var. *pseudotsugae*, is a primary pathogen of Douglas fir, and

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* Infection by rust fungi tends to occur in wave years, or years of high infection incidence between several years of low incidence. This occurs because sporulation, bud burst, and appropriate conditions for spore germination all must coincide for infection to occur.

local epidemics of the disease are associated with forest management practices such as road construction, harvesting, and thinning (Harrington et al., 1983; Hansen et al., 1988). Insect vectors of the disease (bark beetles and root collar weevils) are attracted by compounds released by stressed trees (Harrington et al., 1985), which, together with the tendency to favor Douglas fir in plantation management, has lead to an increase in disease incidence in young plantations (Hansen and Goheen, 1988).

Oak wilt, caused by *Ceratocystis fagacearum*, has caused widespread damage in much of the eastern U.S. The most severe oak wilt epidemics have occurred in managed stands that have reduced species diversity through selective cutting, thinning, and favoring of oak species for regeneration (Wilson, 2001). Further, the introduction of the chestnut blight and Dutch elm disease pathogens eliminated chestnut and elm from eastern forests, and these were replaced by the highly susceptible oaks. Movement of firewood from dead oaks and pruning of trees in urban environments have both contributed to spread of the disease by attracting insects that transmit the fungus to wounds on healthy trees (Wilson, 2001).

42.4.3.5 Foliar Disease Fungi

Foliar pathogens are normally dispersed by air or rain splash and infect foliage of conifers and broadleaf species. Photosynthetic capacity is reduced as a result of infection. Foliar pathogens do not normally kill trees, but can under certain conditions (Agrios, 1997), which include establishment of species outside their normal range, prolonged weather that favors disease development, and establishment of pure, dense stands (Edmonds et al., 2000).

Plantation forestry using exotic species has resulted in increased incidence of several foliar pathogens, including *Dothistroma pini* of radiata pine in New Zealand and *Phaeocryptopus gaumannii* and *Rhabdocline pseudotsugae* in exotic plantations of Douglas fir in the western U.S. (Hood, 1997; Stone, 1997). Native foliar pathogens can also be affected by forest management practices that lead to increased incidence and density of host species. An example from the north-central interior of British Columbia follows.

Dothistroma septospora (*Mycosphaerella pini*) is considered native to Central America and western North America, although it probably originated in South America (Evans, 1984). The fungus is spread primarily through splash dispersal of the conidia and causes a foliar blight on several pine species. Mild summer temperatures and prolonged periods of high humidity or surface water films contribute to serious outbreaks of the disease (Hocking and Etheridge, 1967).

An outbreak of *Dothistroma* is taking place in the interior cedar hemlock zone east of the coast range in northern British Columbia (Woods, 2003). This outbreak is thought to be due in part to weather patterns that have been conducive to spread of the fungus. However, the current outbreak is much larger than what has been observed in the recorded past, and there is good evidence that forest management practices have contributed significantly to the outbreak.

Historically, species composition in the area encompassed by the outbreak was quite diverse, as seen in Figure 42.1 (Woods, 2003). The species compositions of reforested cutblocks are not as diverse (Figure 42.1) for several reasons, including availability of planting stock and government regulations that encourage rapid regeneration of commercial species. Forest management practices, targeted at rapid establishment of preferred commercial species, have dramatically increased host availability and may have effectively removed one of the natural controls of the spread of *Dothistroma* by reducing the species diversity and increasing the incidence of susceptible species.

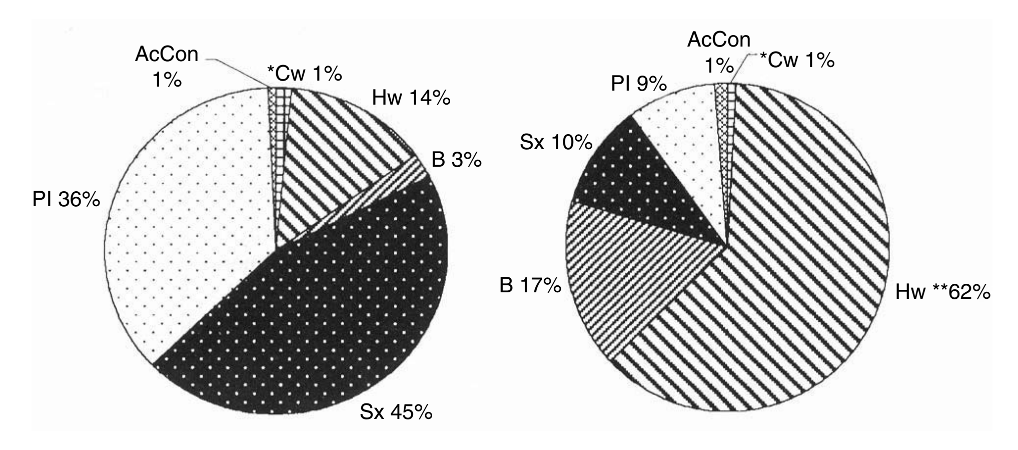


Figure 42.1 Most prevalent species in stands less than 20 years old (left), and in all other stands in the Interior Cedar Hemlock Zone of the Kispiox region. *CW = western red cedar; **HW = western hemlock; B = true firs; Sx = interior spruce; Fl = lodgepole pine; AcCon = black cottonwood. (Adapted from Woods, *Forestry Chronicle,* 79:892–897.)

42.5 CONCLUSION

There are several patterns of impact of forest management that are evident for all three groups of fungi. First, a qualitative shift of the fungal community usually occurs following clear-cut logging. Second, although reductions of diversity have occurred following forest management practices, these effects are usually temporary. Third, disturbances of intermediate intensities may create new habitat conditions, thereby allowing an additional set of organisms to colonize.

The understanding of the relationship between community structure and functioning of the ecosystem is in its infancy with respect to all three fungal trophic levels. A change in forest structure due to forest management has subsequently changed the plant pathogenic fungal community and has resulted in a change of cavity habitat for birds and, thus, a potential change in ecosystem function. In decomposer systems, differences between clear-cut and unharvested forests with respect to saprotrophic fungal community structure did not translate over to microbial processes and metabolic quotients; nevertheless, only one study of which we are aware has fully addressed this relationship. It is unknown whether the ectomycorrhizal fungal community, which establishes after clear-cutting, is better adapted to absorb and translocate nutrients from these soils or whether they are dominant because they are more competitive colonizers of root systems (Jones et al., 2003). Thus, further research, perhaps using new molecular techniques, is necessary to understand the relationship between fungal community structure and function in response to forest management practices with respect to all three fungal trophic levels.

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43

Exotic Species and Fungi: Interactions with Fungal, Plant, and Animal Communities

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43.1 INTRODUCTION

The introduction and spread of exotic and invasive species is considered to be one of the most important problems currently faced in conservation biology. Following habitat destruction, invasive species are often listed among the most pressing threats to biodiversity and ecosystem functioning (Vitousek et al., 1996). The ecological, economic, and social consequences of exotic species have been extensively reviewed (e.g., Bright, 1999; Mack et al., 2000, 2002; Mooney and Hobbs, 2000; Pimentel et al., 2001; Baskin, 2002; Campbell and Schlarbaum, 2002; Meyerson and Reaser, 2003). How do fungi and fungal communities fit into the broader picture of invasion biology? When most people think of exotic and invasive fungi, their first thoughts turn toward destructive plant diseases, such as chestnut blight or Dutch elm disease. But many other fungi with assorted life histories are as likely as plant parasites to have been moved from one geographic location to another. What have been the ecological impacts of these movements of fungal species? Even less attention has been paid to the ecological impact of exotic plant, animal, and microbial species on native fungal species and communities.

In this review, I will first cover definitions of exotic and invasive species. This will be followed by a discussion of the ecology of invasion biology. Much of this information will be taken from the broad literature on exotic and invasive species, but I will use fungal examples where appropriate (I will use a broad definition of fungi that includes oomycetes). Finally, I will examine the ecological impacts of exotic fungi on fungal, plant, and animal communities and the effects of exotic plants, animals, and microbes on fungal communities.

43.2 WHAT ARE EXOTIC AND INVASIVE SPECIES?

In both scientific and popular writings, the terms *exotic* and *invasive* are often used interchangeably. Other common terms often encountered in the literature include *introduced*, *alien*, *nonnative*, *nonindigenous*, *imported*, and *weedy*. But definitions of exotic and invasive species are not necessarily agreed upon by all scientists (e.g., Richardson et al., 2000; Davis and Thompson, 2000, 2001, 2002; Shrader-Frechette, 2001; Rejmánek et al., 2002; see exchange in Daehler, 2001). Many authors do not explicitly define the use of these terms, and this may lead to ambiguities in the literature (Shrader-Frechette, 2001).

The term *exotic* may be best discussed in an evolutionary sense on various spatial and temporal scales. Speciation has a geographic component to it; i.e., species evolve in some geographic location (Losos and Glor, 2003). Over historical time, species geographic ranges will expand or contract through means such as migration or competition. Climatic and geological changes also influence species ranges. However, the term *exotic* has generally been reserved for human movement of a species outside of its native range, thereby accelerating typical biogeographical processes (Mack et al., 2000; Kolar and Lodge, 2001; Lodge and Shrader-Frechette, 2003).

Although often used interchangeably with *exotic*, *invasive* can be seen as more of an ecological term. Some see *invasive* as related to the species in question having some ecosystem impact (Davis et al., 2001). This is the sense of U.S. Executive Order 13112 on invasive species issued by President Clinton in 1999. Other ecologists, however, use the term *invasive* to include any nonindigenous species that has spread and become abundant in a new geographic location regardless of the actual or perceived ecological impact (Daehler, 2001; Kolar and Lodge, 2001; Rejmánek et al., 2002). This point of view suggests that *impact* is too subjective a term to be explicitly used in a definition.

Determining what is an exotic species can be difficult for fungi. Baseline data on the diversity of resident fungal species are limited even for well-studied environments in temperate regions (Hawksworth and Rossman, 1997; Rossman and Farr, 1997; Hawksworth, 2001). Biodiversity studies continue to reveal numerous undescribed species (e.g., Vandenkoornhuyse et al., 2002a, 2002b) or potentially much larger geographic ranges for well-known species (e.g., De Beer et al., 2003). In understudied environments, such as the tropics and the soil, researchers are only now coming to grips with the true amount of fungal diversity. This can lead to potential problems. When undescribed species are found, how likely is it that they are native to that geographic location? In contrast to most terrestrial plant and animal species, fungi are often listed as cosmopolitan, and many are described as having circumglobal geographic ranges. Unlike many plants and animals, fungi are often difficult to detect without a concerted effort using special cultural methods or molecular tools (Miller, 1995). Even for many fungal plant pathogens that are apparently novel and causing major ecological impact, their native range remains unknown (Table 43.1).

While often considered an academic question, species recognition has enormous ecological, economic, and political implications, as governments struggle with establishing quarantines to prevent establishment of unwanted exotics (Palm, 2001; Wingfield et al., 2001). Basic research on species identification and biogeography work is critical for establishing baseline data. Species concepts in the fungi have undergone a dramatic shift from an emphasis on morphology-based species concepts to concepts based on evolutionary biology (Brasier, 1997; Harrington and Rizzo, 1999; Taylor et al., 2000). The use of molecular tools and phylogenetic analysis has allowed for finer distinctions in delimitation of fungal lineages and species recognition. Many fungi thought to represent a single taxon

Table 43.1 Examples of Exotic Fungal Plant Pathogens That Have Become Established in Natural Forest Ecosystems

Pathogen	Disease Name	Host Genus	Indigenous Location	Exotic Location	Reference
Ceratocystis fimbriata var. *platanus*	Sap stain	*Platanus* spp.	Eastern North America	Italy, California	Baker and Harrington, 2001
Cronartium ribicola	White pine blister rust	*Pinus* spp., *Ribes* spp.	Asia	North America, Europe	Smith, 1996
Cryphonectria parasitica	Chestnut blight	*Castanea* spp.	Asia	North America, Europe	Anagnostakis, 1987; Milgroom et al., 1992
Discula destructiva	Dogwood anthracnose	*Cornus* spp.	Unknown	North America	Caetano-Anollés et al., 2001
Fusarium circinatum	Pitch canker	*Pinus* spp.	Mexico, southeastern North America	California	Gordon et al., 2001
Gremmeniella abietina	Sclerroderis canker	*Pinus* spp.	Europe	North America	Sinclair et al., 1987
Lachnellula wilkomannii	Larch canker	*Larix* spp.	Europe	North America	Sinclair et al., 1987
Melampsora larici-populina	Popular rust	*Populus* spp.	Europe	North America	Newcombe et al., 2000
Nectria coccinea var. *faginata*	Beech bark disease	*Fagus* spp.	Europe	North America	Sinclair et al., 1987
Ophiostoma novo-ulmi	Dutch elm disease	*Ulmus* spp.	Asia?	North America, Europe	Brasier and Buck, 2001
Ophiostoma ulmi	Dutch elm disease	*Ulmus* spp.	Asia?	North America, Europe	Brasier and Buck, 2001
Phytophthora cinnamomi	Phytophthora root rot, Jarrah decline	Many hosts	New Guinea?	Australia, Europe, North America	Weste and Marks, 1987; Braiser, 2000; Tainter et al., 2000
P. ramorum	Sudden oak death	Many hosts	Unknown	Western North America, Europe	Rizzo and Garbelotto, 2003
P. lateralis	Port-Orford cedar root disease	*Chamaecyperus*, *Taxus*	Unknown	Western North America	Hansen et al., 2000
Sirococcus clavigignenti-julglandacearum	Butternut canker	*Juglans cinerea*	Unknown	Eastern North America	Furnier et al., 1999

have now been divided into several species (e.g., the *Armillaria mellea* complex) (Harrington and Rizzo, 1999; Taylor et al., 2000; Hawksworth, 2001). To be of use to the study of exotic fungi, species concepts must also be coupled with the study of biogeography (e.g., De Beer et al., 2003). Many fungi once thought to have broad geographic distributions have been shown to have strongly structured populations at scales ranging from landscapes to continents (e.g., O'Donnell et al., 1998; Skovgaard et al., 2002).

In the absence of precise information on geographic ranges, population genetic studies can serve as indirect indicators of newly established species. Founder effects can lead to populations with relatively little genetic variation (Sakai et al., 2001). *Cryphonectria parasitica* and *Fusarium circinatum*, causal agents of chestnut blight and pitch canker of pine, are examples of plant pathogens that have limited genetic variation, based on genotypic and phenotypic markers, in their area of introduction, compared with their native geographic range (Milgroom et al., 1992; Gordon et al., 2001). For other plant pathogenic fungi, such as *Sirococcus clavigignenti-julglandacearum*, *Discula destructiva*, *Phytophthora lateralis*, and *Phytophthora ramorum*, limited genetic variation in the putative exotic location supports the idea of founder effects and recent introduction even though their native geographic range is not known (Table 43.1) (Furnier et al., 1999; Hansen et al., 2000; Caetano-Anollés et al., 2001; Ivors et al., 2004). Examination of the population structure of *Sphaeropsis sapinea*, causal agent of pine tip blight, in South Africa pointed toward multiple introductions of this species rather than a single event (Wingfield et al., 2001).

The continued use of modern population genetic tools and analyses will further refine abilities to test hypotheses on the geographic origin of fungal species and genotypes (Milgroom and Peever, 2003). For example, it has long been suggested that the genotypes of *Phytophthora infestans* that were responsible for the Irish potato famine originated from Mexico (Goodwin et al., 1994). Ristaino et al. (2001) extracted DNA of *P. infestans* from herbarium specimens of infected potatoes collected in Ireland during the mid-1800s. Analysis of mitochondrial haplotypes did not support the Mexican origin of *P. infestans* isolates during this time period (Ristaino et al., 2001). The wide diversity of genotypes of *P. infestans* recently described from the Andean highlands of Ecuador offers additional evidence toward determining an area of origin for this pathogen (Adler et al., 2004).

43.3 ECOLOGICAL AND EVOLUTIONARY ASPECTS OF FUNGAL INVASIONS

One of the main goals in the study of biological invasions is increasing the level of prediction so that prevention and mitigation can be more effectively applied (Kolar and Lodge, 2001). Key questions include: Which species are most likely to become invaders? Which communities are likely to be invaded? What is likely to be the long-term impact of an invader? Numerous papers have focused on developing generalities and models that may be useful in managing the arrival and spread of exotic species (Mack et al., 2000, 2002; Kolar and Lodge, 2001; Rosenzweig, 2001; Sakai et al., 2001; Lee, 2002; Shea and Chesson, 2002).

A biological invasion consists of at least three steps: arrival, establishment, and spread of a novel organism (Liebhold et al., 1995; Mack et al., 2000, 2002). At each step along this continuum fewer potential invading species will survive. Understanding the underlying biology of an invasion requires basic knowledge of life history traits, community ecology, evolutionary biology, and population biology.

43.3.1 Movement and Arrival

Fungi are especially suited for long-distance travel; they are ubiquitous organisms associated with niches ranging from nonliving organic substrates to living plants and animals. Many fungi produce propagules that may be easily moved and are resistant to environmental stresses. Dispersal distances for most fungi are unknown, but many species can be naturally moved over great distances through the air on wind currents (Brown and Hovmøller, 2002; Garrison et al., 2003). But most modern pathways for long-distance movement of fungi are associated with movement of goods and people. Introductions of fungi by humans into new locations may also be deliberate in the case of biological control agents and mycorrhizae (Allen, 1991; Cook, 1993; Ellison and Barreto, 2004; Yourman and Luster, 2004). However, most movement of fungal species is accidental via plants, animals, soil, and packing material (Mack et al., 2002; Campbell and Schlarbaum, 2002). Human movement of fungi is clearly not a new phenomenon. There have been many human migrations throughout time (Diamond, 1998), which have probably moved fungi on or within plant materials and livestock. Many so-called indigenous fungal species have probably been moved at one time or another by humans and are now considered part of the native mycoflora.

43.3.2 Establishment

Once in a new location, a potential invader must establish a viable population. Establishment of introduced species may be evaluated based on the concept of niche opportunity, which defines conditions that promote invasions in terms of resources, natural enemies, the physical environment, interactions between these factors, and the manner in which they vary in time and space (Shea and Chesson, 2002). The likelihood of establishment of exotics depends in part on the number of individuals that are introduced and how often they are introduced (Sakai et al., 2001; Lee, 2002; Mack et al., 2002). Initial introduced populations will be susceptible to demographic, environmental, and other stochastic forces that drive small populations to extinction (Sakai et al., 2001; Lee, 2002; Mack et al., 2002). Individual life history traits that are important for the establishment of fungi include reproductive strategies and genetic variability related to fitness, virulence, and host or substrate compatibility (Mack et al., 2002). Because of their propensity for asexual reproduction, fungi may be more likely to be established in new geographic locations than other organisms that require the movement and introduction of two sexes. In some cases, the availability of suitable vectors may influence the establishment of individual fungal species (Mack et al., 2002; Lachance et al., 2003).

43.3.3 Spread and Invasion

The final step in a biological invasion is spread of the exotic species from the point of its initial establishment to additional locations within its new geographic area (Rejmánek et al., 2002). Many of the biotic and abiotic factors that are important for initial establishment of an exotic organism are also important for the invasion step. Beyond that, there is much uncertainty why some species continue to expand their range. There are potentially many additional factors that lead to invasion, as opposed to simply establishment. Most appear to be associated with the interaction of chance events in the new range, the biological traits of the species, and the invaded community's composition (Mack et al., 2002).

Exotic fungi may become established but not expand beyond the point of initial introduction. *Armillaria mellea*, a common pathogen of woody plants in north temperate forests, was introduced into Cape Town, South Africa, approximately 300 years ago, presumably on citrus planted by European sailors (Coetzee et al., 2001). However, slow

spread of the fungus combined with the eventual urbanization of the area of introduction has restricted the spread of the fungus and prevented it from becoming invasive (Coetzee et al., 2001). Successful invasion may also follow a long lag period (Mack et al., 2000). The lag phase is often seen as an ecological phenomenon as part of an exponential population growth curve. *Entomophaga maimaiga*, a fungal parasite of gypsy moth (*Lymantria dispar*), was released as a biological control of gypsy moth in the 1900s and had a lag period of nearly 70 years before populations increased to the point where it had an appreciable impact on gypsy moth populations (Hajek et al., 1995).

For the most successful exotic species, spread can often be very rapid in contrast to typical patterns of biogeographical range expansion over evolutionary time. A dramatic example of the relative speed between a typical biogeographical range expansion and a human-mediated biological invasion is a comparison of the migration rates of chestnut (*Castanea dentata*) and its exotic pathogen *Castanea parasitica* across eastern North America. Following the last glaciation, chestnut migrated northward from refugia, taking nearly 13,000 years to eventually reach its current geographic range about 2000 years ago (Davis, 1981). In contrast, chestnut blight became established throughout the geographic range of chestnut in less than 50 years (Anagnostakis, 1987, 2001). Similar rapid range expansions of recently introduced fungi in their exotic locations can be documented for many of the other plant pathogens listed in Table 43.1.

Understanding the role of environmental heterogeneity at various spatial and temporal scales has been important in mapping and predicting spread of invasive organisms across a landscape. Climatic heterogeneity over a period of decades has been a major influence on the movement of the white pine blister rust pathogen, *Cronartium ribicola*, southward in the western U.S. (Smith, 1996). At a finer scale, Jules et al. (2002) have documented the interactions of humans and natural landscapes in the spread of *P. lateralis*, the causal agent of Port-Orford cedar root disease, within an individual watershed.

While much literature has focused on invasions as unique biological phenomena, successional and epidemiological theory has much to offer to the study of invasion biology at spatial scales ranging from local to continental (Davis and Thompson, 2000; Mack et al., 2000; Davis et al., 2001; Kolar and Lodge, 2001; Klironomos, 2002; Madden and Van Den Bosch, 2002). For example, recent models have stressed the importance of positive and negative feedback between soil fungi, both mycorrhizal and parasitic, and plants to explain successional patterns and biodiversity (Bever et al., 1997; Hart et al., 2001, 2003). Klironomos (2002) and Callaway et al. (2003) have tested these models with experiments to demonstrate that invasive plant species will have different interactions with native soil fungi than native plant species. These differential responses to fungi may be a major factor in determining invasiveness of individual species. In the case of parasitic fungi, epidemiological theory can be of use to model and predict spread of pathogens through plant and animal populations (Anderson and May, 1986; Campbell and Madden, 1990; Mack et al., 2002). Current models concentrate primarily on annual plants, but have great potential for focusing research and management when applied to invasive species (Madden and Van Den Bosch, 2002).

Evolutionary change in an exotic species may be one of the most important factors leading to invasiveness (Sakai et al., 2001; Lee, 2002). Fungi introduced into new environments will be exposed to new selection pressures that may lead to novel evolutionary outcomes (Brasier, 1995). Rapid evolution of *C. ribicola* has allowed for the pathogen to eventually overcome the occasional resistant genotypes of white pines found in the field (Kinloch and Comstock, 1981). In some instances, movement of novel genotypes of fungi to new geographic areas may be just as problematic as movement of new species. This includes reuniting mating types or introducing particularly virulent strains of parasites. *P.*

infestans existed for over a century as an asexually reproducing species in potato-growing regions around the world; only the A1 mating type was present (Smart and Fry, 2001). The only known area where both mating types existed was in what was considered to be *P. infestans'* native range in Mexico and South America. This changed in the 1980s when the A2 mating type was spread around the world. Sexual reproduction of *P. infestans* introduced more genetic variation into pathogen populations, allowing for adaptation to fungicides and host resistance, as well as production of oospores, a long-term survival structure (Smart and Fry, 2001).

There are a number of other possible outcomes when exotic species or exotic genotypes are brought into contact with indigenous species or other exotic species, ranging from no significant change to hybridization to introgression of individual loci, viruses, and plasmids (Brasier, 2001; Schardl and Craven, 2003). Several recently detected hybridization events have been linked to exotic species (Brasier et al., 1999; Newcombe et al., 2000). A new *Phytophthora* pathogen of alder (*Alnus* spp.) appears to be the result of hybridization between *P. cambivora* and a *P. fragariae*-like species (Brasier et al., 1999). Neither of the putative parent species can infect alder. Host range shifts combining parental host ranges following hybridization have been noted in the rust genus *Melampsora* (Newcombe et al., 2000). Hybridization may also result in the transfer of individual genes rather than the formation of an entire new species. Genetic exchange between *Ophiostoma ulmi* and *Ophiostoma novo-ulmi*, both exotics in Europe, has resulted in the transfer of mating type and vegetative compatibility genes between these two species (Brasier, 2001). These events have allowed *O. novo-ulmi* to become potentially more invasive and destructive.

The ecology of exotic species must also be put into context with other changes in the world's environment (Mooney and Hobbs, 2000; Wardle, 2002). Global climate change may influence the geographic range of already invasive species as well as contribute to the potential establishment of new exotic species. Brasier and Scott (1994) have mapped the potential changes in the geographic range of *Phytophthora cinnamomi* in Europe under scenarios of global warming. This warm-temperature pathogen is predicted to increase its range and influence in the forests of northern Europe, where it is currently absent. Similar changes in geographic range of other fungi may also occur, with changes in precipitation patterns associated with climate change.

43.4 ECOLOGICAL IMPACTS: INTERACTIONS OF EXOTIC ORGANISMS WITH NATIVE PLANT, ANIMAL, AND FUNGAL COMMUNITIES

Ultimately, invasive organisms must interact in some way with native species as they colonize a new ecosystem (Eviner and Chapin, 2003). Quantifying the impacts of exotic species is often impossible or difficult because of a lack of baseline ecological data on invaded ecosystems (Mack et al., 2002; Simberloff, 2002). The ecological effects of exotic species can be measured on different spatial and temporal scales. Exotic species can impact individuals, populations, communities, and ecosystems (Mack et al., 2002). Interactions between exotic and native species may be positive, negative, or neutral (Wardle, 2002; Klironomos, 2002). There may also be cumulative and indirect effects that lead to a cascade of changes throughout an ecosystem (Baskin, 2002; Mack et al., 2002; O'Dowd et al., 2003). Beyond well-known plant and animal pathogens, the impacts of exotic fungi on ecosystem structure and function have not been extensively characterized. This is especially true when we consider potential effects of exotic plants, animals, and microbes on native fungal communities. Communities of plants and animals have been better charac-

terized, and therefore, the effects of exotics species are more obvious and more easily quantified. The limited baseline data on fungal community structure in many ecosystems make the effects of exotics on fungal communities more difficult to discern.

Not all ecologists see every exotic species as having negative consequences and have difficulty using the human constructs of good vs. bad (Rosenzweig, 2001; Slobodkin, 2001). Obviously, not all exotic species cause major ecological and economic damage (e.g., many important agricultural crops). In the case of some deliberately introduced fungi, such as mycorrhizae and some biocontrol agents, the potential benefits may outweigh negatives (Ellison and Barreto, 2004). However, much of this remains untested.

The next sections will examine case studies of the ecological impacts of exotic fungi on plant and animal communities and also the impact of exotic plants, animals, and microbes on native fungal communities.

43.4.1 Exotic Fungal Plant Pathogens

The ecological, economic, and social impacts of exotic fungal plant pathogens have been reviewed many times (e.g., Yardwood, 1983; Palm, 2001, Pimentel et al., 2001; Mack et al., 2002). Because of the well-known impacts of exotic plant pathogens, much effort is made at regional, national, and international levels to restrict movement of plant pathogens in order to protect agricultural crops. However, some of the greatest impacts of invasive plant pathogens have come in natural ecosystems. We can make several important distinctions between exotic fungal pathogens of agricultural crops and natural ecosystems.

Nearly all agricultural food crops can be considered exotic plants depending on where they are grown in the world (Baskin, 2002). For example, potatoes are an exotic species grown all over the world. In North America, major crops such as wheat and rice are native to other parts of the world. In the southern hemisphere, many wood products come from plantations planted with exotic species, primarily *Pinus* and *Eucalyptus* (Wingfield et al., 2001). Many horticultural crops are also exotics. In many agricultural situations, plants are often reunited with pathogens with which they coevolved. Two classic examples include *Phytophthora infestans* (cause of late blight) on potato and *Hemileia vastatrix* (cause of coffee rust) on coffee. In these situations, the host–pathogen system is brought back together under very different environmental conditions (e.g., monocultures, reduced genetic variability in the host, and increased moisture regimes) that may lead to devastating epidemics.

The situation is often different with pathogens introduced into natural ecosystems. In cases where the pathogen and plant host have not coevolved, there is usually limited genetic resistance to infection in host populations (Hansen, 1999; Ennos, 2001). There are many examples of exotic fungal plant pathogens in natural ecosystems, particularly forests (Table 43.1). In some cases, these exotic diseases have nearly eliminated or greatly reduced populations of a single plant species, such as American chestnut due to chestnut blight. In fewer instances, a pathogen with a broad host range has impacted entire forest ecosystems directly. In the jarrah forests of western Australia, *Phytophthora cinnamomi* has a very wide host range and has virtually eliminated most woody plant species over large geographic areas (hundreds of thousands of hectares), converting them to grassland or shrubland, since it was introduced in the 1920s (Weste and Marks, 1987). Recently, *Phytophthora ramorum* has emerged as a presumed exotic with a wide host range that has had large impacts on coastal oak forests in California (Rizzo and Garbelotto, 2003).

Mortality of plants due to exotic pathogens may result in extensive changes in plant community diversity. But the direction of community change is not necessarily predictable. *P. cinnamomi* may cause reduced plant diversity in areas where it attacks and kills the majority of species, as in the jarrah forests of western Australia (Weste and Marks, 1987; Hansen, 1999). On the other hand, *P. cinnamomi* may increase plant diversity in some

forests due to mortality of dominant species. Mortality of shortleaf pine (*Pinus echinata*) caused by *P. cinnamomi* (known as littleleaf disease) has helped to convert seral pine stands into more diverse and disease-resistant late successional hardwood forests in the eastern U.S. (Hansen, 1999). Similar shifts to more diverse forests may also be seen with the decline of the American chestnut as oaks, hickories, and other species have replaced chestnut's position in the forest canopy (Sinclair et al., 1987; Anagnostakis, 2001; Mack et al., 2002). However, this change in forest structure has led to increased susceptibility to other forest insects and pathogens, such as gypsy moth and oak wilt (*Ceratocystis fagacearum*), or multiple causal agent syndromes such as oak decline (Liebhold et al., 1995; Mack et al., 2002; see also below). Continued shifts in species composition mediated by pathogens are expected in these forests (Mack et al., 2002). Epidemics caused by exotic pathogens must eventually be put into context with expected causes and patterns of mortality over long periods to adequately assess their long-term impacts (Rizzo and Garbelotto, 2003). But even following the massive decline of chestnut due to chestnut blight, major ecosystem changes in the hardwood forests of eastern North America have been difficult to quantify because of a lack of baseline ecological data on biodiversity and successional patterns (Simberloff, 2002).

White pine blister rust has received considerable attention for its potential to cause cascading changes in whitebark pine (*Pinus albicaulis*) ecosystems (Tomback and Kendall, 2001). Whitebark pine is a keystone species at high elevations in many locations in western North America. Declines in whitebark pine populations may have adverse effects on animals such as the grizzly bear (*Ursus arctos horribilis*), which uses whitebark pine seeds as an important food source (Tomback and Kendall, 2001). Whitebark pine is also obligately dependent on Clark's nutcracker (*Nucifraga columbiana*) as a means of dispersal. The loss of whitebark pine may cause shifts in foraging behavior of nutcrackers, leading to increased difficulty for recovery and restoration of whitebark pine (Tomback and Kendall, 2001).

The impact of the potential exchange of fungal pathogens between agriculture and native plant communities has not been extensively studied (Gilbert and Hubbell, 1996). For example, the heteroecious rust fungus *Puccinia graminis* is presumed to have been introduced into North America from Europe sometime in the early 1600s (Yardwood, 1983). It has caused devastating epidemics to wheat crops over the past several centuries. But in addition to agricultural cereal crops, dozens of native grass species are listed as hosts in North America (Farr et al., 1989). Has *P. graminis* impacted populations of native grasses? In addition, there are approximately 175 species of *Berbis* (the alternate host for *P. graminis*) native to North America. Although the exotic *Berbis vulgaris* is considered to be the most important alternate host from an epidemiological point of view, there is little to no information on potential impacts on these native hosts.

43.4.1.1 Exotic Fungal Animal Pathogens

Exotic fungal pathogens have been implicated as causes of several animal diseases (Daszak et al., 2000, 2001). *Aphanomyces astaci*, the cause of crayfish plague, was introduced into Europe around 1860 via stocking of North American crayfish (*Pacifastacus leniusculus*) (Alderman and Polglase, 1986). Multiple recent introductions also appear to have been made into the U.K. and Spain (Lilley et al., 1997). This pathogen is associated with high mortality rates and large population declines that threaten some local native European populations of crayfish with extinction (Daszak et al., 2000). In North America, the fungus is endemic and causes a nonlethal enzootic infection. Recent mass mortality and population declines of frogs in many locations around the world have been associated with infection

by a pathogenic chytrid, *Batrachochytrium dendrobatdis* (Berger et al., 1998; Longcore et al., 1999; Daszak et al., 2000). Very low levels of molecular genetic variation among isolates collected from North America, Africa, and Australia support the hypothesis that *B. dendrobatdis* is a recently emerged pathogen in many locations (Morehouse et al., 2003). The exact origin of *B. dendrobatdis* and the reasons for its emergence are still not clear (Daszak et al., 2000; Morehouse et al., 2003).

A number of emerging marine diseases have been associated with fungi (Harvell et al., 1999). A primary example is aspergillosis of corals associated with *Aspergillus sydowii* (Geiser et al., 1998). In many cases, however, it is not known whether these emerging diseases are caused by introduced species or native fungal species whose population dynamics have changed because of changes in the environment. Sources of exotic fungi in marine environments include runoff of silt and pollution from terrestrial environments (Garrison et al., 2003).

43.4.1.2 Exotic Plant Interactions with Native Fungi

The ecological impacts of exotic plants are well known around the world (Baskin, 2002). Exotic plant species have also served as a means for transport of exotic fungal pathogens, mycorrhizal fungi, and endophytes. But interactions of exotic plants with native fungal communities are less well characterized. Native mycorrhizal fungi with broad host ranges (e.g., many arbuscular mycorrhizal fungi) may facilitate invasiveness of exotic plants (Richardson et al., 2000; Bray et al., 2003; Callaway et al., 2003). Endophytes in grasses have been shown to confer herbivore resistance to infected plants. The native endophyte *Neotyphodium coenophialum* may reduce overall plant diversity and enhance invasiveness of tall fescue (*Festuca arundinacea*) in successional fields (Clay and Holah, 1999). The invasive grass *Aegilops triuncialis* (goatgrass) can be facilitated by its interactions with *Ulocladium atrum*, a saprotroph that can occasionally be pathogenic (Eviner and Chapin, 2003). Colonization of goatgrass seed heads by *U. atrum* may allow for more efficient germination by breaking down the woody seed head. Infection of seed heads by *U. atrum* increased goatgrass biomass by 65% compared with plots in which the fungus was not present (Eviner and Chapin, 2003).

Also important are the interactions between fungi and invasive plants that do not occur. In their new environment invasive plants are exposed to many potential fungal pathogens, including above- and belowground organisms. The enemy escape hypothesis suggests that a lack of susceptibility to indigenous pathogens may give an invasive plant a competitive edge over indigenous plant species (Keane and Crawley, 2002; Klironomos, 2002; Wolfe, 2002; Mitchell and Power, 2003; Reinhart et al., 2003; Callaway et al., 2004). For example, in North America the spatial distribution and abundance of native black cherry (*Prunus serotina*) seedlings appear to be controlled to a great extent by host-specific soilborne pathogens, particularly *Pythium* spp., that are associated with adult trees (Packer and Clay, 2000). In northern Europe, where black cherry is considered an invasive species, Reinhart et al. (2003) found that no such inhibition of seedling establishment occurred around adult black cherry trees. Not all plant invasions can be explained by the enemy release hypothesis. Beckstead and Parker (2003) found no such relationships with the invasive grass *Ammophila arenaria*. This plant was found to be impacted by soil pathogens in both its native and introduced locations (Beckstead and Parker, 2003).

Plants are intricately tied into decomposer systems (Wardle, 2002). Invasive plants potentially can disrupt this feedback loop by altering the quantity, quality, and diversity of litter sent to the decomposer subsystem. Bärlocher and Graça (2002) examined the effects of exotic *Eucalyptus globulus* on fungal community structure and function in

streams in Portugal. Aquatic hyphomycete species richness and evenness were significantly lower in streams bordered by eucalyptus than streams bordered by native deciduous forest (chestnut, oak, cherry, and pine) (Bärlocher and Graça, 2002). However, decomposition of eucalyptus leaves and chestnut leaves did not differ between the two groups of streams, although this may have been due to varying chemical and physical properties of the streams as much as to the differing fungal communities (Bärlocher and Graça, 2002). Chauvet et al. (1997) reported similar findings in their comparison of hyphomycete communities in streams bordered by eucalyptus vs. alder (*Alnus* sp.) in Spain.

Introduction of exotic plants may affect the mycorrhizal communities associated with native plant species. In the southern Appalachian Mountains, *Rhododendron maxum* forms dense thickets that reduced the number and changed the composition of mycorrhizal fungi on the roots of hemlock (*Tsuga canadensis*) seedlings (Walker et al., 1999). Species composition of mycorrhizae on hemlock seedlings shifted to *Cenococcum geophilum* from a much more diverse array of species (Walker et al., 1999). The presence of *R. maxum* did not appear to affect the overall diversity of ectomycorrhizal species present in the soil (based on presence of fungal fruiting bodies), but rather impacted the interactions of hemlock seedlings with the mycorrhizal community (Walker and Miller, 2002).

43.4.1.3 Exotic Animal Interactions with Native Fungi

Animals may indirectly impact fungal communities by affecting the resources available to fungi (Wardle, 2002). Wardle et al. (2001) used a series of exclosures in various plant communities to examine the impacts of exotic browsing mammals on above- and belowground food webs in New Zealand. They found that the effects of grazing varied considerably depending on the ecosystem in question. Impacts of grazing on aboveground food webs and soil macrofauna were mostly negative. However, aboveground grazing effects on microbial soil food webs could be positive or negative. In this case, microbial biomass was taken as a whole rather than divided into functional groups. Positive effects on some microbial groups may come from additions of urine and dung into ecosystems or through the reduction of organisms at higher trophic levels, e.g., fungal grazers. Activity of the exotic earthworm, *Dendrobaena octaedra*, has been shown to reduce densities and community composition of microfungi in soils in both mesocosm and field studies in North America (McClean and Parkinson, 1998, 2000). Invasions by earthworms favored the growth of faster-growing fungal taxa at the expense of slower-growing taxa. In hardwood forests of central New York, invasion by the exotic earthworms *Lumbricus rubellus*, *Lumbricus terrestris*, and *Octolasion tyrteum* were correlated with reductions in colonization by arbuscular mycorrhizal fungi on sugar maple (*Acer saccharum*) (Lawrence et al., 2003). Physical disruption of mycelia and responses to increased nutrient availability due to earthworm activity were two mechanisms suggested to explain mycorrhizal suppression by earthworms (Lawrence et al., 2003).

Defoliation of trees has been shown to influence mycorrhizal community structure (Gehring and Whitham, 1991, 1994; Cullings et al., 2001). These studies with native defoliating insects suggest the possibility of impacts on mycorrhizal communities through introductions of exotic defoliating insects such as the gypsy moth. Exotic insects may also interact with native fungi in an indirect but positive direction. The beech scale insect, *Cryptococcus fagisuga*, was introduced into North America in the late 1800s (Mack et al., 2002). This insect creates feeding wounds that serve as infection points for an exotic canker-causing fungus, *Nectria coccinea* var. *faginata*. However, a native canker fungus, *Nectria galligena*, may also benefit from the feeding wounds in some geographic areas (Sinclair et al., 1987). The gypsy moth has also been a major contributor to oak decline

in eastern North America. Stress caused by defoliation of oaks by gypsy moth often leads to increased colonization by indigenous fungal pathogens such as *Armillaria* spp. and *Hypoxylon* spp. (Sinclair et al., 1987; Mack et al., 2002).

The introduction of the yellow crazy ant, *Anoplolepis gracilipes*, to Christmas Island, a tropical island in the Indian Ocean, set off a series of events over many decades that included changing of fungal communities (O'Dowd et al., 2003). New mutualistic relationships between the ants and honeydew-secreting scale insects have led to increases in the prevalence of sooty mold fungi (*Capnodiaceae*), which in turn reduces photosynthesis leading to dieback of canopy trees (O'Dowd et al., 2003).

Exotic animals may serve as vectors for fungal pathogens of indigenous plants and animals (Feio et al., 1999; Kiesecker et al., 2001; Martin and Dale, 2001). Studies have suggested that *Saprolegnia ferax*, a pathogen of amphibians, is greater in toad populations exposed to hatchery-reared trout (Kiesecker et al., 2001). *Basidiobolus ranarum* is a zygomycetous fungus that causes basidiobolomycosis in humans, horses, and other vertebrates. Recent studies have suggested that exotic reptiles may serve as vectors for this fungus (Feio et al., 1999). The European elm bark beetle, *Scolytus multistriatus*, was introduced into North America several decades prior to the introduction of *Ophiostoma ulmi* and is the dominant vector of this pathogen in many places in North America (Sinclair et al., 1987).

43.4.1.4 Exotic Fungi and Other Microbes Interactions with Native Fungi

Much research on exotic plants and animals has been directed at competitive interactions at the same trophic level. Invasions by related species may disrupt the composition and functioning of entire communities (Sanders et al., 2003). With fungi, most research has been conducted on the effects of the exotic fungus on other trophic levels, i.e., plant and animal pathogens. How have exotic fungi and other microbes interacted with native fungal communities? This is probably the least studied area concerning exotic fungi.

There are many examples of introductions of exotic nonparasitic fungi, but their ecological impacts have not been quantified and may not be apparent because of a lack of baseline data. Most have probably been brought in on plant material and include mycorrhizal fungi, endophytes, and saprobes. Species lists of agarics of islands, such as Hawaii or New Zealand, contain numerous examples of exotic species (Horne, 2000; Hemmes and Desjardin, 2002). Approximately 88% of agarics listed by Hemmes and Desjardin (2002) for Hawaii were considered to have been brought in by humans over the past 1500 years. Macrofungi, such as *Amanita phalloides* (Tanghe and Simons, 1973) and *Clathrus archeri* (Arora and Burk, 1982), have long been considered to be exotic in North America, but their ecological interactions with native vegetation have not been explored. Many nonpathogenic microfungi have probably been introduced on exotic plants and animals. For example, *Ophiostoma piceae* is considered a nonpathogenic blue-stain fungus that has been introduced from the northern hemisphere to many parts of the southern hemisphere on exotic pines and other conifers (Harrington et al., 2001; De Beer et al., 2003).

In contrast to fungal plant pathogens, human movement of fungal mutualists, primarily mycorrhizal fungi, has been encouraged in many places. Many species of mycorrhizal fungi have been introduced along with exotic conifers and eucalyptus around the world (e.g., Galán and Moreno, 1998; Fogel and States, 2001). Introductions of mycorrhizal fungi are often necessary for the establishment of plantations of these tree species; however, exotic fungi may also facilitate the invasiveness of exotic tree species beyond the initial plantations (Richardson et al., 2000).

Chapela et al. (2001) describe a situation in exotic *Pinus radiata* plantations established in the Pármo grasslands of Ecuador in which *Suillus luteus* is associated with carbon depletion in the plantations and surrounding grassland. *Pisolithus* spp. have been deliberately introduced around the world as part of plantation establishment (Allen, 1991; Dell et al., 2002). This cosmopolitan genus was traditionally considered monospecific and most isolates classified as *P. tinctorius* (Martin et al., 2002). However, recent work supports strong geographic separation of *Pisolithus* lineages and therefore raises the probability of a number of different species (Martin et al., 2002). These different lineages have long been considered to differ in their ecology and host specificity (Dell et al., 2002; Martin et al., 2002). This suggests the introduction of novel species and genotypes of *Pisolithus* to many parts of the world. However, the long-term impacts of these introductions on native plant species as well as other fungi has not been adequately explored.

Although it concerns two exotic species, the study of the interactions between *Ophiostoma novo-ulmi* and *Ophiostoma ulmi* may offer insights into what may potentially occur with the introduction of a fungus that has a similar niche to an already established species. In both North America and Europe, the more aggressive *O. novo-ulmi* appears to have quickly replaced *O. ulmi*, a species that had been established several decades prior (Brasier and Buck, 2001). The mass mortality of chestnut due to chestnut blight most likely has had an impact on decomposer community structure and function in the affected forests. Some fungal species will be lost due to the demise of chestnuts, while others will be increased at least temporarily because of the influx of dead material into the decomposer system (Baxter and Gill, 1931). As described above, still other fungal species may be enhanced in the long run by shifts in the species composition of plant and animal communities.

Phytophthora ramorum is now a common foliar pathogen of many woody plant species in the coastal forests of California (Rizzo and Garbelotto, 2003). In the course of studying this emerging and presumed exotic pathogen, at least one apparently native foliar *Phytophthora* species (*P. nemorosa*) was discovered (Hansen et al., 2003). Populations of *P. nemorosa* tend to be greater outside of areas where *P. ramorum* has invaded. Is *P. ramorum* replacing these native foliar pathogens? Other exotic foliar pathogens, such as *Discula destructiva*, have surely interacted with indigenous foliar fungi, including saprobes, endophytes, and other parasites.

Other microbes (e.g., bacteria, viruses, phytoplasmas) may also play direct or indirect roles in impacting native fungal communities, although these have not been quantified. Exotic pathogens that significantly reduce populations of herbivores may have a major impact on plant community structure (Dobson and Crawley, 1994). For example, the rinderpest virus was introduced into native grazing communities of Africa by infected cattle. The subsequent population crash of native grazers, such as impala, has led to massive changes in plant community structure, with woody plants (primarily *Acacia*) becoming dominant in former grasslands (Dobson and Crawley, 1994). Similar changes in plant communities have been documented with the introduction of the *Myxoma* virus into exotic rabbit populations in Great Britain (Dobson and Crawley, 1994). In this case, a lack of rabbit herbivory led to conversions of grasslands to oak woodland. Changes in saprobic and mycorrhizal fungal communities during typical plant community succession have been documented (Allen, 1991; Frankland, 1998), and it is highly likely that exotic herbivore-mediated successional changes would result in changes in fungal communities.

HIV, causal agent of AIDS, may be considered an introduced virus in most places in the world (Mayer, 2000). HIV infection has predisposed humans to fungal pathogens such as *Pneumocystis carinii*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Candida albicans*, and *Penicillium marneffei* (Clark and Hajjeh, 2002; Woods, 2002). Many of these fungal pathogens may be considered indigenous, but rarely cause deadly mycoses

except in immunocompromised patients. Because of intensive treatment, invasive mycoses are becoming less important in developed countries, but they still cause considerable mortality in AIDS patients in developing countries (Clark and Hajjeh, 2002).

43.5 CONCLUSIONS

This chapter has dealt with biological aspects of exotic and invasive species. But there are also political and economic considerations in any discussion of exotic species. Global trade continues to increase along with the movement of plants, animals, fungi, and other microbes. Based on World Trade Organization (WTO) guidelines, restrictions on international trade to prevent introduction of novel organisms must be science based (Palm, 1999). Basic research on fungi can direct the establishment of quarantines (Palm, 1999, 2001; Wingfield et al., 2001). However, major gaps remain in our knowledge of the diversity and ecology of most fungi, and because of this, there are often difficulties in developing political solutions to invasive species.

Gathering of baseline biological information is critical for use in risk analysis. Understanding of fungal biodiversity and fungal community structure, including links of fungal communities with other organisms, is needed before we can truly determine the ecological impacts of biological invasions. New molecular methodologies that allow for characterization of fungal species composition in understudied habitats, such as soil and within leaves and roots, have revealed incredible amounts of species diversity (e.g., Arnold et al., 2001; Hawksworth, 2001; Helgason et al., 2002; Vandenkoornhuyse et al., 2002a, 2002b). Much recent work on species concepts, population biology, and biogeography must now be incorporated into regulatory thinking (C. Brasier, personal communication).

The ecological impacts of exotic species on native plant communities have been studied for many destructive plant pathogens. However, there have been relatively few studies examining the effects of exotic species on native fungal communities. With a few exceptions (e.g., Wardle et al., 2001; Bärlocher and Graça, 2002), most research has been carried out at the level of interactions between individual species and not at the fungal community level. Studies of exotics must also be carried out along with other fungal ecology research, not considered separately. We must also recognize that the biology of organisms in their native environment may be different than in their place of introduction. This has been especially true for plant pathogens that may be more destructive in different environments. The more we begin to understand fungal ecology in general, the better we will be able to predict the ecological trajectories of future introductions.

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Section 4

Preserving Fungal Communities

44

Fungal Conservation: Some Impressions — A Personal View

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44.1 INTRODUCTION: WHY IS IT IMPORTANT TO CONSERVE FUNGI?

Fungi are vital to the process by which available nutrients from dead organismal material are recycled to be made available to new plants and animals, moving these through deserts of poor nutrition, and passing them onto the dominant ecosystem components in exchange for organic compounds. Without these organisms, life as we know it would be very different indeed. In addition, they are enjoyed by many people as part of the landscape, and along with many birds and small animals, humans include them as component to their diets. Without fungi, whole ecosystems would fail or be replaced by very poor substitutes. But despite fungi being so important, they are forgotten in everyday conservation policies. Effective conservation depends on high degrees of knowledge of the biology, especially ecology, of the individual fungal species. This is generally not available, and when it is, it is not in the depth necessary to formulate useful rules.

44.2 HOW DO WE CONSERVE FUNGI?

Probably an additional reason for fungi being left out of conservation strategies is that myco-conservation is easier said than done. The most important single factor is to preserve the habitat, viz., the conditions and places in which fungi live. If habitats are destroyed or damaged by pollution, then the fungi are lost.

Rare or uncommon fungi often coincide with rare habitats that may be protected under other legislative powers, but in the U.K., where the mycota is comparatively well known, fungi are rarely incorporated into the management schedules for these sites; there are, in fact, very few nature reserves anywhere in the world that have been designed solely for fungi. Conservation attention, even by mycologists, is restricted to the larger fungi or macromycetes, which is an unspecific grouping dependent solely on whether the fungus can be seen with the unaided eye. It, therefore, covers a whole spectrum of taxonomic groupings although it is true that most belong to the basidiomycetes. This in itself is a great admission to leave out several thousands of microspecies, but that is our state of knowledge and alas is a failing of this presentation also.

There are now 40 species of nonlichenized larger fungi listed under the U.K. Biodiversity Action Plan (BAP), and four species have been given special legal protection under U.K. law through Schedule 8 of the Wildlife and Countryside Act–1981. Seventeen British species of larger fungi are also on the European list as agreed at the Bern Convention; they range from the rare *Amanita friabilis* with few British records and *Gomphus clavatus*, which has not been seen in Britain for many years, to the fairly widespread bolete relative, *Phylloporus pelleterii*, with areas of good distribution, and *Hohenbuehelia culmicola*, found in *Ammophiila* beds along the eastern seaboard of the British Isles. The Republic of Ireland has a similar, although not as extensive, list of Bern species in need of attention, and mycologists from Eire are in close collaboration with their British colleagues. These species are given protection under European Community laws; other European countries have similar or even larger numbers listed. In Britain this is in marked contrast to the conservation of bryophytes, vascular plants, and lichenized fungi, which number in their hundreds for protection. This scenario is similarly reflected throughout Europe, but when the approximately 450,000 estimated number of larger fungi worldwide is considered, this worrying phenonemon is not only patently absurd but ecologically a disaster. However, luckily there are improvements in most European countries, and it is hoped that from lessons learned, useful policies can be exported to those desparately in need of guidance in the developing countries.

Most sites that are well managed for their birds, mammals, invertebrates, or plants are likely to possess a rich mycota, and so in such cases, no special action is generally necessary, but this is not the case for many sites rich in fungi. Indeed, it is neccessary to seek mycologists' advice on the fungi in a selected site, as their presence is dependent on the appearance of the fruiting structures, and these often appear only sporadically. If they do not appear at a site for 1, 2, or 3 years, is the fungus threatened or, if more, can it be considered extinct? Or are the fruiting structures just hidden from sight in the soil in the vegetative state? After all, the collector has to be at the site of fruiting when sporomes occur, and it is well known that such structures can disappear very rapidly — and therefore miss being seen. There are records of species of fungi in both undisturbed tropical and temperate areas that have not appeared at a site for a generation or more; they have undoubtedly been there all the time. Even after logging, many stress-tolerant fungi are able to persist for very long periods without fruiting (Dahlberg and Stenstrom, 1991). The more frequently one can visit a site to conduct a survey, and continue over as long a period of time as feasible, the better; only then will it be possible to gain a fairly accurate idea of a site's potential (Watling, 1995; Straatsma and Krisai-Greilhuber, 2003). At least molecular techniques have come to our aid, and these are now being applied to locate, in the absence of the sporomes, fungi in the soil for conservation needs. Because some fungi have entered the political agenda in several European countries by being incorporated into legislation, they have attracted financial support for research that would not have been otherwise available. For instance, the setting up of the national U.K.

BAP program (Fleming, 2001; Duckworth et al., 2002), and its subsequent effect in local government arenas, means that there is a greater awareness of fungi in the academic and public domains, and some of the thorny questions of fungal ecology are now being addressed in a scientific way. Thus, the Earth Summit (United Nations Conference on Environment and Development) held in 1992 in Rio de Janeiro and the signing of the Rio Convention have directly stimulated an interest in fungi.

Conservation managers, however, can only make decisions on the data that are made available to them, so recording is still fundamental and necessary. Luckily, throughout Europe there are sites that have been studied by individual mycologists or small groups of mycologists for long periods, generating published local fungus floras (mycotas), which can offer some guidance in assessing the potential of a site, but such studies are limited. There are few sites that can boast long-term and frequent monitoring; such sites are known, for instance, in as widely separated sites as northwestern North America (Norvell, 1995) and the Borders of Scotland (Watling, 2002), where a site of 7.5 ha has accrued 9500 records of fungi and data on springtails, mites, nematodes, etc., gleaned from monthly visits in November–April and July, bimonthly visits in May and June, and generally once a week in August, September, and October. There are others elsewhere in Europe, e.g., Austria (Straatsma and Krisai-Greilhuber, 2003) and the Netherlands, where currently 566 plots distributed over the country are under regular surveliance.

44.3 ADDITIONAL THREATS

The general threat to fungi, other than habitat loss or modification to the water table and its interlinked factors, is pollution. Atmospheric and chemical pollution or nitrogenous enrichment may all lead to a decline in fungal fruiting as admirably demonstrated (e.g., Gulden and Hanssen, 1992), particularly in the Netherlands (Arnolds, 1985). Individually, we can do very little about general atmospheric pollution, even though it is seen to be potentially harmful; many ecto- or sheathing mycorrhizal fungi associated with arborescent plants are particularly susceptible, expressed as a dysfunction in the balance between the plant and the biotroph. Such inbalance can lead to disruption of plant growth, and debilitation leads to greater suceptiblity to pathogenic attack and interest from invertebrate herbivores, inevitably causing decline; an excellent example has been demonstrated in the Black Forest region of Austria, with decline in the health of the spruce forests (e.g., de Wet et al., 2003). These effects on both natural forests and those planted by man, the latter hoping to receive a financial return, can be very destructive.

Many modern fungicides are designed to kill fungi and are often broad spectrum; in parallel to fertilizer seepage or drift from farmland, such chemicals can be detrimental. Thus, the management of woods adjacent to arable land requires active liason between farmer and landowner in order to reduce the effect of pesticides, fertilizers, and any other chemical additives (Borges and Rotheroe, 2002). With this in mind, Plantlife International and its collaborators recently produced a brochure for U.K. landowners to manage their land "with fungi in mind" (Anon., 2002).

Unfortunately, to conserve a particular group of fungi may not agree with the policy for conserving a special plant or animal; a compromise, a search for a way around, or a pragmatic decision has to be taken. Equally it is not always possible to manage a property for all fungi, as ectomycorrhizal species often require different parameters to saprotrophic elements. What is necessary is to try and retain as much of the mycodiversity in the ecosytem as possible.

44.4 VEGETATION TYPES: WOODLANDS

In woodlands, the dominant trees, certainly in temperate and northern countries, the rain forests of Malaysia and Central Africa, and the eucalypt forests of Australia, are ectomycorrhizal and, therefore, depend on the interplay of a suite of larger fungi, the components of which may change over the history of the woodland. Successions of species have been demonstrated in Europe (Last et al., 1987) and under planatation conditons in other parts of the world, or an increase reported in the overall number of species recorded as the wood matures. Even in communities that are initially considered stable, such as the Cameroon *Berlinia/Tetraberlinia* rain forest, a gradual increase over time in the size and number of individuals in the groves of these caesalpinoid legumes is apparent; similar changes have been recorded by Henkel (2003) for *Dicymbe* woodland in Guyana. These are both ectomycorrhizal communities, and the pattern of the soil mycota within them therefore must change. In contrast, in the chronosequence of dunes on the coasts of southeastern Queensland, new plant communities have formed in relatively recent geologic times and are now accompanied by a range of fungi, including ectomycorrhizals (Watling and Li, 1999).

Some species of fungi are undoubtedly characteristic of old-growth forest, whereas others are only found with young plants. A mosaic of woodland types, whenever possible, is therefore recommended. The present European Agricultural Set-Aside Policy, where arable land is returned to woodland, will undoubtedly offer many habitats for fungal colonization and can only be good for the general biodiversity of an area. The fungal inhabitants will no doubt come in naturally as either wind-borne spores or vegetative propagules, or be transported by small mammals such as voles and mice or even larger ones such as deer. In the case of plantation trees, many of the fungi have entered directly from the nursery that was used as the source of planting material. Sometimes in such cases, species may become dominant where they would not naturally occur, e.g., *Suillus flavidus* seen in plantations in Shetland (Watling, 1992). This species has a restricted range in the U.K., even in mainland Scotland, where it occurs in the remnants of the Caledonian pinewoods. The Shetland trees were grown in nurseries close to the natural habitats for this species, and it is hypothesized that the association was made there. In the pine woods (*Pinus radiata*) of Australia, several boreal fungi dominate the visible mycota, e.g., *Lactarius deliciosus* and *Rhizopogon luteolus*, and even in the Falkland Islands, isolated in the southern Atlantic Ocean, the truffle *Hydnangium carneum* has been found under eucalypts. Some of these associations formed in the ameliorated conditions of the nursery may find themselves ousted by more aggressive species from the local mycota.

The discussion so far has dealt with only one component of the forest ecosystem, viz., sheathing mycorrhizals, but the other important fungal components in such communities are the saprotrophs. Some fungi, through their ability to rot wood, can hollow out old trees. The fissures, nooks, and crannies that are so produced are known to encourage nesting birds, roosting bats, and other nocturnal small mammals, or mammals and invertebrates that may hide during the day from predators. Nutrient flow is increased around such hollow trees from the droppings of the animals. This can be especially important in the tropics, where the soils are often extremely thin; such fluxes are undoubtedly a very useful nutrient source in an otherwise rather restricted nutrient environment. Such hollowed trees are widespread under the prevalent humid conditions. In Europe, similar old trees are often termed *veteran trees* (Anon., 2000) and, however misshapen, are a very important component of the landscape, and their protection on any property should be of high prority.

Other fungi grow on limbs both while attached to the tree and when fallen to the forest floor. While some fungi attack branches of diameters of the order of a human wrist,

others colonize twiggy and leafy material, even specializing in the parts of the leaf on which they fruit — the lamina, the vein, the petiole, etc. Thus, many different substrate resources exist in a woodland. Relatively recently a totally new fungal ecosystem has been demonstrated, first in New and then Old World rain forests, but now known to be present in modified form in suitable places in the boreal region, e.g., the damp Atlantic woods of the British Isles. This is where fungi in the canopy glue twiggy and leafy material together, so preventing it from falling to the forest floor. In this way soil fungi are withheld from this rich food resource. Fungi may also colonize limbs high in the upper story where their activities are not seen until the limb is damaged or dies. Such fungi are acting initially as endophytes and may only fruit when the woody debris falls to the forest floor, but at least they have the advantage of being within the food resource before confronting colonization from soilborne fungi.

To encourage the conservation of woodland fungi, any site must be maintained as an entity with a mosaic of tree ages and with a closed canopy, if that is what the site has developed over the years. It is, however, often forgotten by conservationists and naturalists that many ecosytems rich in fungi have, in fact, been established by human activity in distant, yet historic time; this must always be borne in mind in formulating any conservation policy. This is more obvious in the temperate areas of the world, but even in the tropics personal experience has reminded me that things are not always as they first seem. The high level of mycodiversity in logged-over rain forest in Penninsula Malaysia has as much to do with the previous logging regimes and the history of occupation as with the fungal components. Even countries termed *developing* in modern economic jargon have often had a long history of agriculture and animal husbandry, sometimes long since vanished, yet the effects have become part of the landscape. In Finland, Ohenja (1988) has examined the fruiting patterns of larger fungi in respect to woodland management now and in the past; such studies can identify very important conservation management tools.

Scarce woodland types wherever they occur should be at the top of any shopping list for conservation and maintained as the existing stand type. It is true, in parallel to the logged-over Malaysian forest, that many plantations in temperate areas have a much higher visible biodiversity than more natural communities, but this can be very misleading. This means that it is important for a specialist to assess the fungal species present because high diversity of fungal fruiting may not necessarily mean a potentially important conservation area.

Undoubtedly, in the future it will be necessary to recognize the importance of sites on both national and international scales. Thus, the old Caledonain pine forest is a rare ecosystem in the British Isles and has for Britain some very rare, attendant fungi, species that in Scandinavia are common and widespread. Similarly, the montane woods of dwarf willow, topping many of the Scottish, Lake District, and Welsh mountains, especially those with Least Willow, *Salix herbacea*, are also a rarity in the U.K., unlike their counterparts in Scandinavia and the Alps, and are home to many infrequently recorded British fungi. The Atlantic hazel woods, in contrast, are unique in Europe and are of international importance for not only fungi but also lichens and bryophytes.

Long-established conservation activities and woodland policies will have to be changed if fungi are to be included in the future and their management intertwined with the history of the site, as this is generally what has produced the unique sites seen today. In large areas of planted trees, corridors of native vegetation linking pockets of similar communities undoubtedly assist in mantaining a rich biodiversity in what otherwise might be considered a desert of uniformity (Alexander and Watling, 1987). The corridors allow movement between refugia of plant diaspores, animals, and birds, the last two carrying spores among their fur and feathers and in their guts, although it is known that fungi can

persist in small isolated communities for long periods. A dedicated policy maintaining such patterns can only assist conservation.

Fire is an important part of many woodland ecosystems in the world, and some fungi have adapted to this process, but burning brash simply to remove dead wood is folly and should not be encouraged. Dead wood is a good resource for many fungi, and habitats will be rapidly lost if the wood is removed; such removal also reduces the occurrence of moisture reservoirs after drought (Amaranthus et al., 1989). Certainly, in the U.K. specialized fungi of bonfire sites and burnt shrubs, the latter possibly brought there by attracted beetles, are known and may fruit almost immediately or soon after fire, but they are transitional.

In Australia, however, several fungi are encouraged to fruit after bush fires from specialized subterranean structures, but in general we have little knowledge of what role burning plays in the initiation of fruiting, and certainly nothing substantial on what happens to the general population of mycelial fungi in the soil (Pilz and Perry, 1984; Jonsson et al., 1999). There is evidence from northern Europe that controlled, prescribed burning in woods certainly favors fruiting of several lignicoles (Lindmeyer and Franklin, 2002).

In the U.K., a number of wood-decaying fungi have been selected for conservation, e.g., *Piptoporus* (= *Buglossoporus quercinus*) (Roberts, 2002) and *Hericium* spp. (Boddy and Wald, 2003). Such fungi are dependent on a continuous supply of dead or old trees. Dead wood in old-growth forest in both northern Europe (Parmasto and Parmasto, 1997) and northwestern U.S. supports some fungi of very restricted range that demand conservation consideration (Castellano et al., 1999, 2003). Only now is the importance of rotten wood being fully appreciated in conservation terms, and dead wood should be incorporated into management programs whenever possible; such a substrate is also attractive to invertebrates. Translocation of dead wood might be a consideration in special cases, but conditions should be kept as close to the original as possible when giving a helping hand. Too many woods in Europe, especially Britain, are "vacuum cleaned" for aesthetic considerations, and public education would go some way to change attitudes. In other countries, however, wood is collected in rural and tribal communities as a source of firewood for cooking. Thus, in Zambia fuel collection and the production of charcoal have created vast areas of open woodland called miombo. These are certainly recognizable and characteristic, yet undescribed species in such communities, often referrable to unique infrageneric taxa, are found. What their rarity status and uniqueness is, and their importance to this woodland community, can only be guessed, although they are collected for food (Buyck, 1994; Härkönen et al., 1995).

Judicious clearing of invasive, nonnative plants from woodland may be a possible approach in selected areas and an acceptable procedure in certain well-defined circumstances. In a U.K. nationwide survey of stipitate thelephoraceous hydnoid fungi, one of the activities in the north of the British Isles was to map the distribution and habitat preferences of basidiomes (Newton et al., 2002a, 2002b). This has led to a focused research program to answer central questions such as whether these fungi exist in superficially similar areas in which they have not been seen to fruit. In contrast, the same national survey in the south of England encouraged the removal of invasive rhododendrons (*Rhododendron ponticum* — native to central and southern Portugal and southern Spain and Asia Minor) from a site known previously to support basidiomes, but where they had not occurred for some years (Marren, 2000). In order to cause as little damage to the ecosystem by trampling and disturbance, the rhododendrons were cut and removed by hand and a small hand winch was used to extract stumps. Fruiting has now resumed in the managed areas. The clearing of the litter layer in some Dutch plantations has also had positive effects for the fruiting of ectomycorrhizal fungi. It is believed that damaging pollutants are sequestrated in the litter layer and released slowly, but constantly, over the years, damaging the root sheaths

(Barr and Kuyper, 1993). Additional work has been conducted on the effects of forest thinning on sporome production (Pilz and Perry, 1984; Colgan et al., 1999). The research grants that the U.K. studies on hydnoid fungi have attracted have been a direct result of the U.K. BAP program and have allowed studies on related fungi, e.g., the exceedingly rare *Boletopsis leucomelaneum*, to be conducted in tandem. Marrying or merging several similar projects together makes sense and uses the finances more efficiently and effectively.

The northern spotted owl (*Strix nebulosa*) is the top of the food chain in the old-growth forests of northwest U.S.; it feeds on voles, ground squirrels, etc. (Maser et al., 1978). In turn, the small prey mammals, judging from the fungal spores in their stomach contents, rely on fungi as a very important component of their diet. Many of these fungi, especially the sequestrate species, are associated in ectomycorrhizal relationships with the dominant trees in the old growth (Waters et al., 1997). The fungi also find themselves a source of food for many invertebrates in both their fruiting and vegetative phases. Thus, there is a reticulate pattern of interconnections. The owl has attracted finances for conservation studies, and the spin-off is the realization that the fungi are as important to its continual existence as everything else. The results have been published as *Handbooks to Strategy*, two beautifully illustrated volumes dealing with how to collect, identify, survey, and handle those fungi considered important to this ecosytem and termed *ROD species* (record of decision) (Castellano et al., 1999, 2003).

The close relationship between fauna and mycota, from the author's observations, operates in other parts of the world, ranging from the rain forests of West Africa, Southeast Asia, and the eucalypt forests of Australia to the long, modified landscapes of Europe, all of which support such patterns. Stomach contents of red squirrels in Scotland, for instance, in the 13 samples examined, contain the spores of both epigeous fungi, e.g., *Leccinum* and hypogeous species, such as *Melanogaster ambiguus* (Turnbull, 1995).

44.5 GRASSLANDS

Grasslands are generally maintained by grazing or fire. Unlike in other parts of the world, in Britain only the former operates, although maintenance of heathland, a rather widespread plant community, especially in Scotland and northern England, does incorporate burning regimes. Grassland burning may be deliberate or natural, and both have led to some important species groupings. The degree of grazing is very important: good management can maintain a rich diversity of fungi, while overgrazing can destroy whole communities (Marren, 2001). An increase in stocking levels for greater financial return can be achieved by increasing the availability and quality of the food for the animals, that is, by increasing the productivity of grasses achievable by application of fertilizer. Such additives appear to be detrimental to most naturally occurring fungal communities.

While dunging is a natural phenonemon, too much nitrogenous input from overstocking is highly deleterious, and the effects become equable to organic and inorganic additives applied directly to the soil surface. In addition, the lush growth of vascular plants that inevitably results inhibits fruiting. At the moment, there is little or no work on the effects of the application of vermicides, etc., to domesticated animals, on the quality of the dung, but what has been done does not support loss of species; the jury is still out at the moment. However, there is some work that is offering pointers to the effects of soil additives on fungal fruiting; at Sourhope in the Borders of Scotland, experimental plots have been studied for many years by soil scientists and have been reexamined over several seasons by a team of mycologists (Deacon, 2001) with rather surprising results, the validity of which are being statistically analyzed.

Natural soils are usually deficient in nitrogen and phosphorus, and the presence of good fruiting of certain fungi indicates a pristine, unimproved sward; application of lime can be deleterious and, if found necessary, should be done with great caution. Some grasslands miss conservation attention because they are acid communities and poor in associated vascular plants and animals although they may be rich in the basidiomycetous wax caps (*Hygrocybe* spp. <H>), entolomataceous fungi (members of the agaric family Entolomataceae <E>), clavarioids (*Clavaria* sp. and *Clavulinopsis* sp. <C>), and the ascomycetous earth tongues (members of the family Geoglossaceae <G>). Grasslands are the first community to which a formula has been devised to give some indication as to the quality of a site. On the basis of high scorings, grasslands can then be designated as important either nationally or internationally. This formula is calculated from the numbers of species of each group of fungi noted above (CHEG) and, although a rather rough guide, is undergoing statistical adjustment and refinement. On this basis, many of the untreated grasslands of Scotland are seen to be internationally important for their wax cap mycota (approximately 90 sites), some boasting 25 or more different species of *Hygrocybe* alone. This is in sharp contrast to other areas in Europe, e.g., Denmark (Boertmann, 1995), where such communities are fast declining; Scotland, therefore, has an obligation to conserve a representative sample of these communities.

The formula has been provisionally modified with the inclusion of members of the genus *Dermoloma* (D) and *Porpoloma metapodia* (P), but this is unwarranted because, at the moment, extensive studies subjected to statistical analysis would appear to indicate that there is little correlation between the main units (Newton et al., 2003). To complicate it still further is not advisable at this juncture. These initial results for Scotland indicate an unsuspected rich source, and priorities must now be taken into account as to the other organisms present at these sites, human activites, amenity factors, etc., as it would not be feasible financially to conserve all sites.

Alas, countrywide improvement of these communities by application of fertilizer is unfortunately still continuing as a means of increasing productivity, and this appears also to damage the bryophyte flora; all good wax cap sites have been demonstrated to have a strongly developed moss layer. There have been suspicions that there is an unproven link between this group of larger fungi and mosses, something that needs to be examined in greater detail; in the meantime, moss killers should be avoided at all costs and a healthy moss layer encouraged. Certainly with some ascomycetous disc fungi such connections are now well founded. By targeting the wax cap mycota (Anon., 2001c), which is only one component, be it colorful, it has been possible to sweep in other groups such that meaningful qualitative data have been accumulated, which in turn have lent themselves to quantitative analysis. This has driven experimentation. With an increase in public knowledge, there is a subsequent effect that slowly releases government finances for research on which all good conservation policy should be based. Plantlife International has produced two brochures, one on the distribution of the pink wax cap (*Hygrocybe calyptriformis*) (Anon. 2001b) and one, more recently, on the management of grasslands for wax caps (Anon., 2003). Such exercises are important to make progress in conservation. Factors that need to be in place for management of grasslands for the conservation of wax caps are cessation of ploughing, change of the field drainage, nonremoval of topsoil with or without reseeding, and no application of additional dung or soil additives, including artificial fertilizer; any mowing should be accompanied by removal of the hay produced. Elimination of stock equally can have damaging effects, as this leads to encroachment by woody scrub, which finally swamps the grassland and inhibits the fruiting of meadow fungi. Establishing woodland is more attractive than maintaining undisturbed grassland because government subsidies offset the costs of planting trees, making it financially rewarding. Undoubtedly, there is a link between increasing subsidies and the loss of fungal diversity.

44.6 ARTIFICIAL GRASSLAND COMMUNITIES AND PICKING FOR FOOD

In Europe, outstanding fungal sites are often lawns of old country houses and churchyards, and fungi are frequently associated with off-green golf courses; all are stable and often hundreds of years old. Persuasion through dialogue toward a compromise in management for these ancient features must be conducted; otherwise, mismanagement can soon lead to a downward spiral. Although animal and plant components might superficially appear unchanged, and therefore do not signal disaster, many fungal species are more sensitive. Some of the best sites in Europe for wax caps are called parklands, where established grassland is associated with groups or scattered, single, often ancient trees. They are grazed by sheep or horses and, in many estates, by introduced deer; cattle are less frequently used and perhaps are not the best grazing beasts for a fungus site, as they tend to trample the soil and produce waterlogging. However, there is evidence that some species, e.g., *Microglossum olivaceum*, are favored by at least some trampling to the soil.

The parklands are a marriage of grassland and trees in juxtaposition, and they support grassland fungi as well as woodland and woodland margin species, including many ectomycorrhizal species. Because these estates were landscaped many generations ago, the trees are now coming to a precarious part of their life and, although alive, are prone to windthrow, etc. Management should include a vigorous replacement policy to ensure a continuum of the association and a mosaic landscape.

More natural woodlands with a well-established grass sward beneath exist elsewhere, particularly in warmer localities, and resemble, except in their vastness, the ancient parkland of rural England. Thus, the miombo woodland of Central and East Africa and the *Pinus kesiya and Pinus merkusii* woodlands of the Phillipines are examples from very different climates and parts of the world. In the former, zebu cattle graze the often very coarse grass, which is periodically burnt to encourage the growth of more succulent leafage, and in the latter, water buffalo are used with only occasional, accidental burning. These have been long-established traditions and are associated with important fungi; indeed, in many parts of Africa these woodlands supply edible species as indicated earlier.

Collecting fungi for food, especially if commercially exploited, is frowned upon by general conservationists in northwestern European countries, but collecting in general is the least of the worries for conservation of fungi and pales into insignificance when habitats are lost and plant communities changed out of recognition by the application of additives, land mismanagement, and poor silviculture. It is true that trampling and the associated soil compaction are hazards, but by the protection of valuable areas and the adoption of a code of conduct, the problems can be alleviated. Different European countries have different policies on collecting wild mushrooms. In both England and Scotland guidelines have been issued (Anon., 1998a, 1998b; Watling and Ward, 2003). Research, which is unique in this area in the U.K., has shown in Scotland, at least, that at present levels mushroom picking is a sustainable activity (Dyke and Newton, 1999; Dyke, 2001); the results parallel those from North America (Norvell, 1995).

44.7 MARITIME, RIVARINE, AND WETLANDS; HEATHS

Coastal grasslands apparently support fungi that are adapted to or able to withstand an exposed environment (Rotheroe, 2001); in some areas and in northern Scotland, the grasses and forbs are augmented with dwarf tree species such as the creeping willow, *Salix repens.* This introduces an ectomycorrhizal component to the mycota similar to that seen in

parklands discussed above (Watling, 1981). Generally, coastal grasslands are more fragile than inland communities, as they are often on skeletal soils, although some, such as the machair of the Western Isles of Scotland, are extremely ancient farming systems. Maritime grasslands generally suffer more easily from overgrazing and human activity, leading to extensive wind erosion, scouring out the sands down to the water table and producing wet areas called slacks. Some of these latter areas in western Europe become stabilized from colonization by willows and alders, and new communities are so developed. In the Cooloola sand mass in southeast Queensland, Australia, parallel changes have taken place and rain forest has developed within the dune system, supporting diverse mycota, flora, and fauna. In these widely disparate communities, one of the biggest threats to the community is from nutrient-enriched runoff from adjacent areas of agriculture. Again, this is where liason with land managers is necessary and realignment of production subsidies with conservation aims is required.

With the threat of forthcoming rising sea level, it has been important to record the fungi of sand dunes. Now that this has been completed for a wide section of the British coast (Rotheroe, 1993), it is possible to incorporate the information gleaned into a robust conservation policy. Sand dunes have long been stabilized by the introduction of exotic species; *Hippophaë* or pines have been used in many parts of Europe. Many of these vascular plants have been accompanied by their own attendant mycota.

Acid heaths are hardly impressive for their biodiversity, although they may be spectacular as landscapes and support rare or uncommon nesting birds. Many species of fungi are found under these conditions, and examples may be found in the blanket bogs of the boreal areas, especially where there are dwarf shrubs incorporated, and on the hilltops in the tropics, such as those of Penninsula Malaysia and Borneo. One species of such boggy areas that demands special attention in Britain through a BAP program is *Armillaria ectypa*, a ringless relative of the honey fungus; it grows among rushes on wet boggy areas and is at present under intensive study by mycologists at the University of London.

44.8 PROGRESS

Conservation in Europe was formalized at the European Congress in Oslo in 1975, and what started as a handful of participating countries has spread far and wide. Since then, there have been several publications on fungal conservation, including the recent volume by the British Mycological Society dedicated to conservation (Moore, 2001); in this volume Moore describes the development of fungal conservation in the U.K. The 1992 European Mycological Congress held at Kew departed from the format of previous congress programs by introducing a specific theme for the meeting: “Examining, Investigation, Recording and Conservation of Fungi.” Its proceedings published a year later bring together researches and views held at the time. A symposium on conservation was also held during the International Mycological Congress in Oslo in 2002 calling on speakers from widely separated countries. So fungal conservation has come of age. However, one vociferous comment in Oslo was that not enough has been done for fungal conservation and not enough lobbying has been undertaken on fungi’s behalf. This is not strictly true as the Oslo session demonstrated, but it must be admitted that there is still a long way to go. There is little doubt that in the U.K., the introduction of a list of common names by Plantlife International and collaborators will make political candidates, elder statesmen, and the general public more aware and able to better handle fungi in the future. Other European countries and the U.S. do not suffer from lack of such a list, and many names

are regularly used by the general populace; in non-European countries a vast array of common names are often found (e.g., Härkönen et al., 2003).

Certainly in the U.K. fungi are at last being featured in government legislation and are being incorporated into national conservation policy; several publications have resulted from sponsorship, e.g., English Nature, on *Piptoporus quercinus*, stipitate hydnoid fungi and *Hericium* spp., including *Creolophus*; Scottish Natural Heritage–Fungi: Naturally Scottish (Watling and Ward, 2003), hydnoid Thelephoraceae (Newton et al., 2002a, 2002b) and Hygrophoraceae (Newton et al., 2003); and Countryside Council for Wales, grassland fungi (Anon., 2001a).

Many countries in Europe have produced lists of fungi that are thought to be extinct, threatened, or need urgent or some conservation. Such compilations are called Red Data Lists, and these are now being taken seriously by governments, although to different degrees by different official bodies. A European Red Data List is being prepared (Ing, 1993). Some of these Red Data Lists include several hundred threatened taxa. Some countries, e.g., U.K., are improving their preliminary lists (Ing, 1992) after the gathering of more reliable data while other countries, e.g., Sweden, are revising their earlier versions and homing in on particular taxa or habitats (Nitare, 1998). A country's possession of a Red Data List is important as a political statement; although politicians and civil servants nod their heads in approval as to all of the uses of fungi in everyday life, it is a name on a Red Data List that has international implications. Indicator species have not been given the attention they deserve in the U.K., but have been successfully used in the study of old-growth forests in the northwestern U.S. The Swedish government, in underpinning its Joint Environmental and Forestry Policy in Conservation of Biodiversity, published an illustrated account of selected cryptogams. Indeed, rare and endangered fungi and their environments figure strongly in the book (Nitare, 2002). Some fungi are usually associated with special features of a habitat and only occur in the remnants of former important vegetational types. Such species, when used in Swedish conservation, are termed *signal species* and at present number 130. Data sheets are provided for these in addition to 55 bryophytes and 87 lichenized fungi. In Malaysia, Lee et al. (1996) have explored the possible use of ectomycorrhizal fungi as potential bioindicators.

Rather ambitious mapping schemes have been developed in Europe and published as atlases; the Dutch Mycological Society has led the world in this field (Nauta and Vellinga, 1995). The British Mycological Society possesses a huge database of records from its forays, and individuals are now contributing many records (Minter, 1986), in addition to the data from the two government research programs on wax caps and stipitate hydnoid fungi. The information gathered by just these two societies (Nauta and Jalink, 2001) has been used to provide an idea of the distribution of selected species of fungi and a basis for the monitoring and production of management plans on several threatened species. Other countries in Scandinavia and western Europe possess parallel information. The Fungimap scheme in Australia is helping mycologists in this vast island continent collect baseline information, some of which has already been used to assist in the conservation of rare mammals (May, 2002). This project calls on a whole range of mycologists, mostly amateurs from all the states of Australia. Many of these schemes are now on the World Wide Web. *Fungi Canadensis*, authored by Scott Redhead, documents the larger fungi of Canada. The fact sheets are based in design on those produced for an array of microfungi by the former Commonwealth Mycological Institute (now CABI) as phytopathiological maps. The maps are useful summaries of available information on selected microfungi, but can be incorporated into any wider study because sooner or later an attempt at incorporating microfungi in conservation schemes is inevitable (Borges, 2002). At least there appears to be a very close relationship between microfungus and plant host, which is a useful beginning.

What is still lacking are real guidelines that can be offered to those who actually have to ascertain the priorities in conservation and those who implement them on the ground. A start has been made with the publication of popular leaflets in several countries. The U.K. Fungus Conservation Forum, consisting of representatives of interested bodies and individuals, was founded in 2000 by Plantlife International (then Plantlife) and facilitates the pooling of resources and pinpoints areas of activity, especially to encourage research to address specific questions. Linked with agencies entrusted with British Conservation (English Nature, Scottish Natural Heritage, Countryside Council for Wales, Northern Ireland Environmental and Heritage Service) and the research councils (Natural Environment, and Biotechnology and Biological), national and local government schemes have acted as springboards for research. Through such, fungal conservation is given much needed respectability and credibility, often necessary even in persuading our fellow biologists.

Mycologists are still required to be something between scientist and naturalist. The difficulty of developing sound methods of reproducing field data is because fungi are sporadic in their appearance, and when the sporomes appear, one invariably has to resort to microscopic examination to identify the species involved. This has led many people, including biologists in other disciplines, to consider fungi as nothing more than background noise, with probably little real effect on the total ecology of a site. Others argue that fungi produce so many spores anyway that they do not need conservation because they can look after themselves. To the initiated, these arguments are patently untrue; widespread education is required. But if this is the view in Western culture, how much more difficult will it be to convince developing countries? There is much to do and so little time in which to do it.

If nothing else, we do have reliable fungal lists for many areas in Europe compiled from diligent collecting over generations by interested amateurs (Minter, 1986; Nauta and Jalink, 2001). It would be a fitting tribute to these volunteer workers for their studies to go some way toward a better understanding of fungal distribution and conservation. Their work has led to the identification of some rather special sites that are extremely rich in fungi. A list of such hot spots for fungi has now been drawn up for the U.K. by Plantlife International (Anon., 2001b), and although only a first attempt, some measure has been made at ascertaining future conservation measures that might be necessary. This is a broadbrush approach, but it does indicate the dedication and determination of those working in the area.

Internationally, the International Union for Conservation of Nature and Natural Resources (IUCN) is the oldest world conservation body, joining 150 or so countries; powerful and well organized, it seeks to influence and encourage the conservation of integrity and diversity of nature and ensure that use of natural resources is equable and ecologically sustainable. IUCN has taken on board fungi, although relatively recently, and there is a specialist group for fungi within the Species Survival Commission (Courtecuisse, 2001; Perini, 2002). Alas, it is starved of funds to seriously address conservation strategies and promote conservation actions for threatened fungi, as the group competes with the more showy vascular plants, furry mammals, and feathered birds. Presently, it is attempting to test and improve the Red Data criteria and categories. The total worldwide challenge is an enormous responsibility, and this has taken higher priority through the recent launch of the Global Strategy for Plant Conservation, in which fungi are an integral part.

Fungi are invisible for much of their life cycle, but we owe members of this group of organisms, better known as the fifth kingdom, a great debt for maintaining the health of the world's ecosystems, and especially the microfungi, given little attention above, for supplying many important pharmaceuticals and food additives. What is needed is a general management manual for rangers, nature wardens, etc. Some have attempted to make a

start; it is hoped that this will be followed (Arnolds, 2001; Borges and Rotheroe, 2002; Watling, 2003) and that the above review will assist in this respect.

ACKNOWLEDGMENTS

There are too many people to list individually, but I thank all who have exchanged reprints and reports with me and with whom I have had extremely useful discussions; they know who they are, and most are reflected in the reference list. I particularly thank the Plantlife International team for their enthusiasm and dedication to the conservation of fungi, especially Deborah Long, Stirling, Scotland, and Edinburgh, and Stephen Ward, formerly of Scottish Natural Heritage, who have done so much toward fungal conservation in Scotland.

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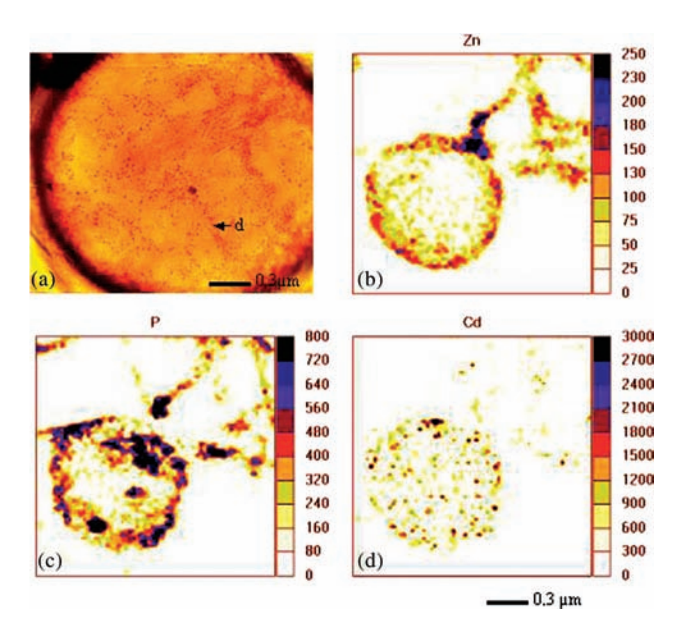


Figure 14.2 Metal localization in spores of *Glomus* sp. (a) Spore stained with sodium rhodizonate, suggesting the deposition (d) of heavy metals on the inner surface of the cell wall. (From Turnau, *Acta Soc. Bot. Pol.*, 67, 105–113, 1998.) (b–d) PIXE elemental maps of spore isolated from polluted soil; concentrations given in mg kg^{-1} (Turnau, Mesjasz-Przybyowicz, and Przybyowicz, unpublished material).

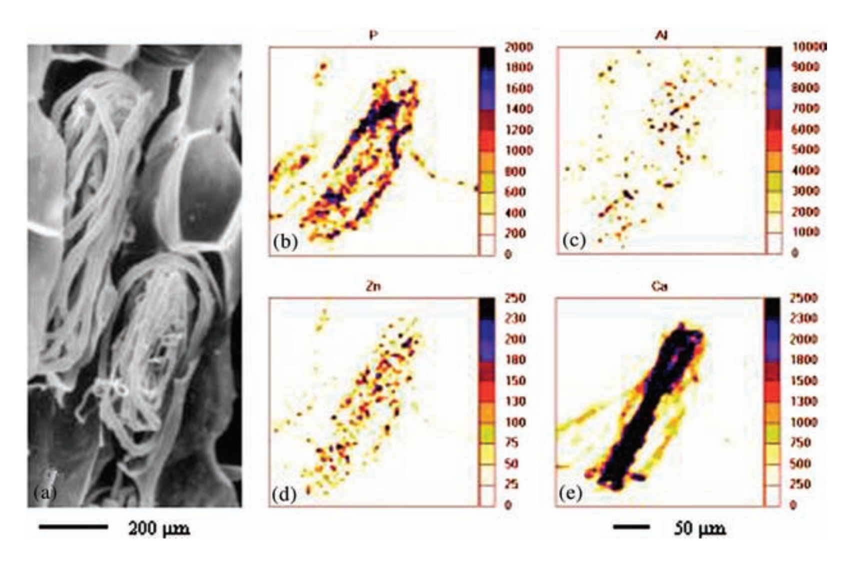


Figure 14.3 Element distribution in orchid mycorrhizas. (A) SEM micrograph of fungal coils. (B–E) PIXE elemental maps of coils separated from the plant material; concentrations given in mg kg^{-1} (Turnau, Mesjasz-Przybyowicz, and Przybyowicz, unpublished maps; for more information, see Jurkiewicz et al., 2001).

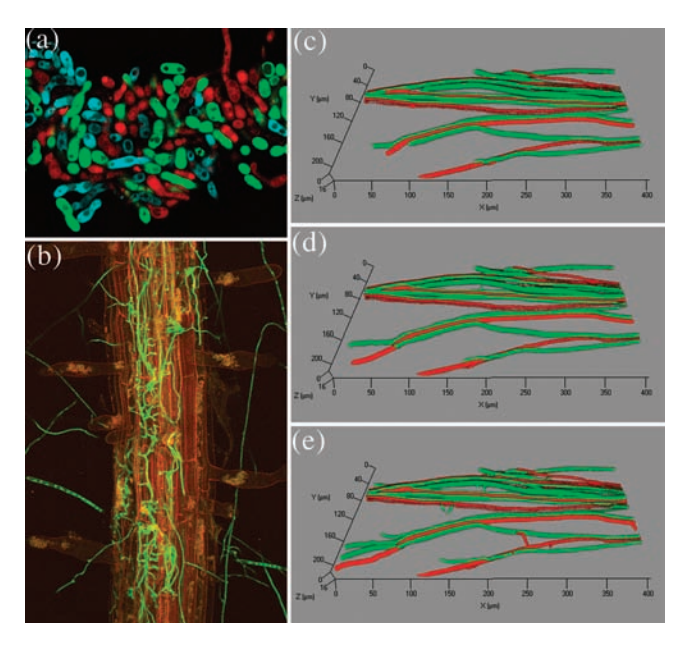


Figure 15.7 (a) Spectral confocal techniques allowed clear separation of closely overlapping fluorescent molecules such as AmCyan (blue), ZsGreen (green), and ZsYellow (red) following linear unmixing. (Images provided courtesy of K. Czymmek, T. Bourett, J. Sweigard, and R. Howard.) (b) *In vivo* constitutive cytoplasmic expression of the reef coral fluorescent protein ZsGreen in *Fusarium oxysporum* was used to monitor disease progression in *Arabidopsis* during root infection. (Images provided courtesy of K. Czymmek, J. Sweigard, M. Fogg, and S. Kang.) (c–e) Four-dimensional (three-dimensional over time) series of cytoplasma expressing ZsGreen and AsRed *Fusarium* hyphae as they interact in culture. These three-dimensional stacks were selected from a four-dimensional data set (T = 0, 32, and 72 min) that monitored hyphal growth every 8 min over a 3-h period. (Images provided courtesy of V. Cooke and K. Czymmek.)

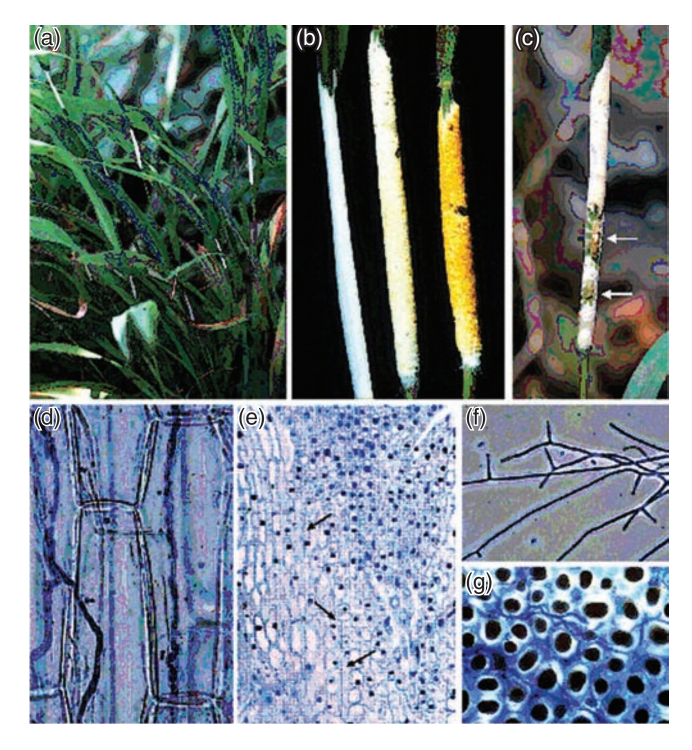


Figure 24.2 Coordinated life cycles of *Epichloë* spp. and their grass hosts. Pleiotropic symbionts undergo both life cycles on different tillers, whereas other *Epichloë* species may not permit seed set and are thus obligately sexual and horizontally transmitted as indicated at right. The asexual species (*Neotyphodium* spp.) undergo only the vertical transmission cycle indicated at left.

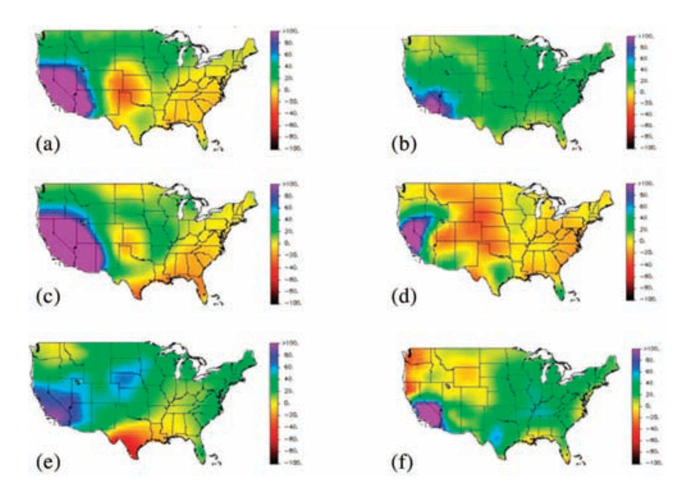


Figure 33.4 The extent to which soil nutrient variables, edaphic factors, soil moisture, and season impact soil fungal functional diversity within the five vegetation zones along the Pine Canyon Watershed in Big Bend National Park, Chihuahuan Desert as visualized using an RPA biplot. Data were grouped by years (1999, 2000, and 2001) and by season, summer (August) and winter (January). Site codes are LDS = lowland desert scrub; CR = creosotebush bajada; SG = sotol-grassland; OF = closed canopy oak forest; OP = high elevation oak–pine forest $N0_3$ = extractable NO_3–N; NH_4 = extractable NH_4–N; moisture = soil moisture; SOM = soil organic matter as loss on ignition. Data modified from Sobek and Zak (2003).

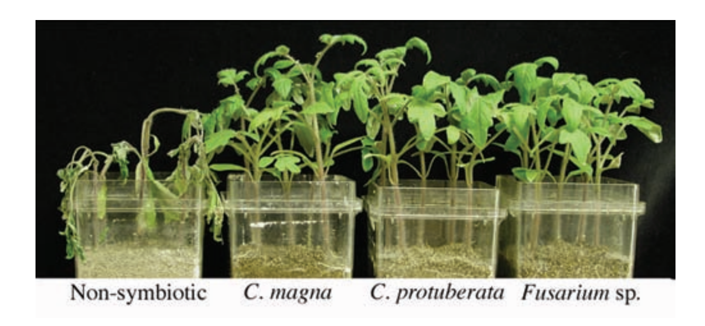


Figure 34.1 Nonsymbiotic and symbiotic plants colonized with the indicated endophytes were grown in sand for 2 weeks with adequate watering. Watering was then stopped and plants left to dry. Nonsymbiotic plants wilted after 4 days of desiccation, while the symbiotic plants stayed hydrated for 9 days before wilting.

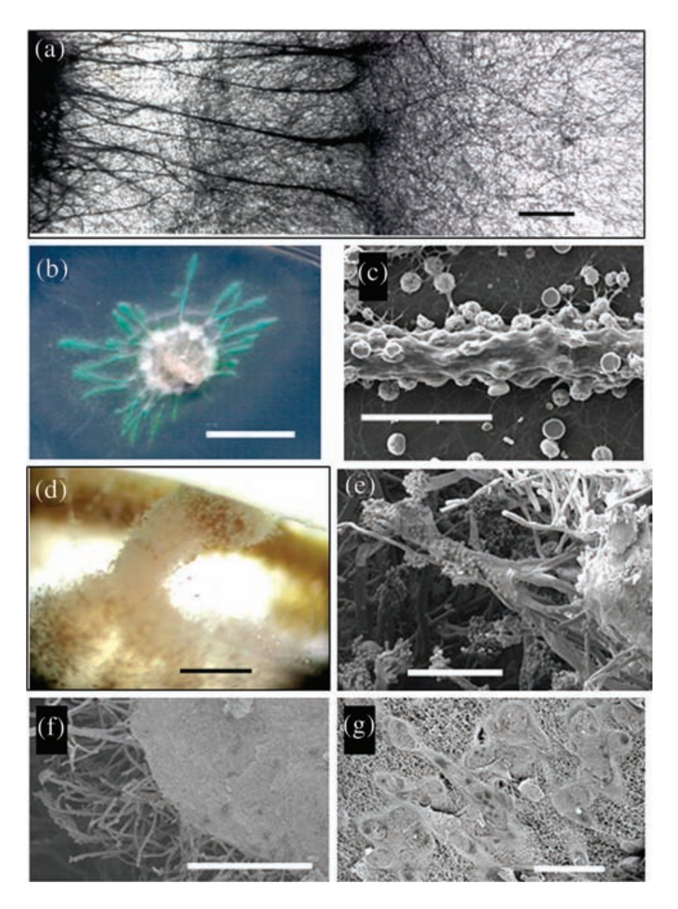


Figure 37.5 Unusual structures of aggregated and longitudinally aligned hyphae formed by fungi in response to toxic metal stress. (a) *Cladosporium cladosporioides*: melanized strands formed in the gap between an inoculum domain (Czapek–Doz agar tile) and a metal-containing tile (1 m*M* Sr and 5 gl^{-1} sucrose) on the right-hand side of the image. Bar = 0.5 mm. (b, c) *Beauveria caledonica*: strands formed on modified Melin–Norkrans (MMN) medium containing copper phosphate, covered with copper oxalate crystals. (b) LM image of the colony on the membrane. Bar =10 mm. (c) A strand with crystals (Au/Pd-coated air-dried sample). Bar = 200 μm. (d–g) *Aspergillus flavipes*: large synnema (= coremium) covered with conidiophores and synnema, formed between an inoculum domain (Czapek–Doz agar tile) and a metal domain (1 m*M* Cu gl^{-1} sucrose) on the top right-hand side of the image. (d) LM image of large synnema. Bar = 1mm. (e) Cryo-FESEM image of synnema on the large synnema surface. Bar = 50 μm. (f, g) Cryo-FESEM images of cross-fractured large synnema showing compact biomineralized hyphae inside with conidiophores and synnema in the outer shell. Bar = 100 μm (f) and 10 μm (g). (Fomina and Gadd, unpublished.)

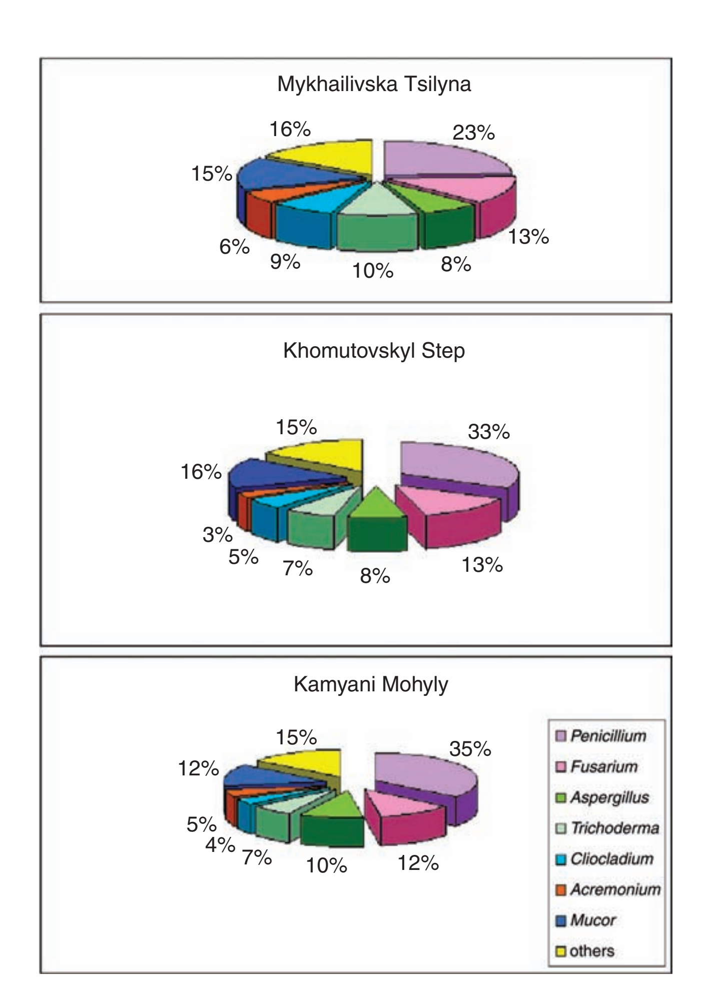


Figure 40.1 Correlation of dominating groups of soil micromycetes in Ukrainian Nature Steppe Reserve. The list of *Aspergillus* species had only one additional member — *A. ustus* (Bain.) Thom et Church. The following species were not found in the Khomutovskyi Step: *Botrytis cinerea* Pers.: Fr., *Gliocladium catenulatum*, *Humicola grisea* Traaen, and *Scopulariopsis brevicaulis* (Sacc.) Bainie; however, they were isolated from Mykhailivska Tsilyna soil. The share of melanin-containing species was 17.8%. Among the species that were rare in Khomutovskyi Step, it is important to mention *P. dierckxii* Biourge and *P. vulpinum* Seif. et Samson in addition to the above-mentioned fungi. *Fusarium oxysporum*, *Gliocladium roseum*, *Trichoderma viride*, and *Penicillium raciborskii* were dominant fungi (see Figure 40.2).

Biological Sciences

The Fungal Community: Its Organization and Role in the Ecosystem, Third Edition addresses many of the questions related to the observations, characterizations, and functional attributes of fungal assemblages and their interaction with the environment and other organisms. This edition promotes awareness of the functional methods of classification over taxonomic methods, and approaches the concept of fungal communities from an ecological perspective, rather than from a fungicentric view. It has expanded to examine issues of global and local biodiversity, the problems associated with exotic species, and the debate concerning diversity and function.

The third edition also focuses on current ecological discussions — diversity and function, scaling issues, disturbance, and invasive species — from a fungal perspective. In order to address these concepts, the book examines the appropriate techniques to identify fungi, calculate their abundance, determine their associations among themselves and other organisms, and measure their individual and community functions. This book explains attempts to scale these measures from the microscopic cell level through local, landscape, and ecosystem levels. The totality of the ideas, methods, and results presented by the contributing authors points to the future direction of mycology.

Features:

- Bridges the gaps between ecological concepts and mycology
- Provides insight into the complexity of studying fungal communities and the importance they may have on broad, ecosystem, landscape, and local scales
- Promotes the future study of new aspects of mycology, with the contributing authors often including controversial, thought-provoking material
- Offers a framework for integrating fungal activity over spatial and temporal scales of resolution
- Demonstrates new molecular and microscopic methods for analyzing fungal structure and function



